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DISTRIBUTION OF LIPASE IN THE TISSUES UNDER NORMAL AND UNDER PATHOLOGIC CONDITIONS

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THIS paper gives a description of pictures obtained in various organs and tissues with my technic for the microtechnical visualization of sites of lipase activity.[1] The theoretic background of the method was given in the original paper.

MATERIAL AND TECHNIC

A number of normal tissues and organs of the following species were examined: man, rhesus monkey, dog, rabbit, guinea pig and rat. The pathologic material consisted partly of surgical specimens, partly of autopsy material.

The original technic was used in this work except for minor modifications and the introduction of a new substrate in addition to the previous ones. It was found that tissue blocks infiltrated with acetylcellulose before paraffin embedding offer definite advantages. This substance largely eliminates shrinkage; it keeps the sections, which otherwise have a tendency to break up into pieces when floated on lukewarm water, firmly together; it also obviates the necessity of protecting them with a layer of collodion before staining. Nitrocellulose is unsuitable for this purpose because, owing to its solubility in a mixture of acetone and benzene, most of it will be removed from the tissue block when it is transferred from acetone to benzene. The maleate buffer recommended in the original paper can be dispensed with altogether, and, instead, the incubating mixture can be neutralized with tenth-normal sodium hydroxide to p_H 6.8 to 7.4, bromthymol blue being used as an indicator. The new substrate is "Product 81", a stearic ester of comparatively short-chained polyglycols, manufactured by the Onyx Oil and Chemical Co., Jersey City, N. J.

The modified technic is as follows:

1. Fix thin slices of tissue in chilled acetone for twelve to twenty-four hours in the ice box. Fresh tissues are preferable, although entirely satisfactory results can be obtained with tissues kept unfixed at ice box temperature as long as twenty-four hours.

2. Dehydrate in two changes of absolute acetone, twelve to twenty-four hours each, at room temperature.

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1. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **58**:362, 1945.

3. Impregnate in a 5 per cent solution of acetylcellulose in acetone for twenty-four hours. Eastman's cellulose acetate "high acetyl, low viscosity, no. 4644" was found satisfactory.

4. Drain pieces and transfer them into two changes of benzene, one hour each.

5. Embed in paraffin at a temperature of 56 to 62 C., using two changes, one to one and one-half hours each.

6. Cut sections at 4 to 8 microns, float them on lukewarm (± 35 C.) water and mount on slides.

7. Run slides through xylene and graded alcohols to distilled water.

8. Incubate at 37 C. for six to twelve hours in the following mixture:

Stock solution I:

Glycerin	150 cc,
10 per cent calcium chloride	50 cc. each,
Half-molar maleate buffer p_H 7 to 7.4	50 cc. each,
Distilled water to make	1,000 cc.

Stock solution II:

Five per cent aqueous solution of Tween 40 or Tween 60 (obtainable from Atlas Powder Co., Wilmington, Del.) or of Product 81.

With merthiolate added in a concentration of about 0.02 per cent, both stock solutions keep in the ice box for months.

Before use, add 2 cc. of stock solution II to 50 cc. of stock solution I. The maleate buffer is prepared by dissolving 5.8 Gm. of maleic acid in a mixture of 94 cc. of normal (4 per cent) sodium hydroxide and 6 cc. of distilled water. As mentioned, it can be left out entirely, in which case the mixed incubating solution must be neutralized to the p_H specified.

9. Rinse slides in distilled water.

10. Transfer slides to a 1 to 2 per cent solution of lead nitrate for ten to fifteen minutes.

11. Rinse slides thoroughly in repeated changes of distilled water.

12. Transfer slides for one minute to a dilute solution of light yellow ammonium sulfide (a few drops to a Coplin jarful of distilled water). Sites of lipase activity will show up in a dark brown shade.

13. Wash under the tap. Counterstain with hematoxylin and, very lightly, with eosin.

14. Dehydrate in graded alcohols. Clear in gasoline or tetrachloroethylene (perchloroethylene). Mount in clarite dissolved in the same solvents. Do not use toluene or xylene, which will cause gradual fading of sections.

The method is reliable, not a single failure having been observed in working up over 100 cases. Its only shortcoming is an occasional failure to obtain good nuclear staining with hematoxylin, especially after the use of the Tweens, much more rarely with Product no. 81. The reason for this phenomenon is not understood; it may happen that of two consecutive serial sections treated in an identical way, one will show excellent, and the other very poor, nuclear staining.

Confusion may arise from the presence of dark brown pigments, such as melanin, lipochrome or hemosiderin, or of granular deposits of calcium salts, which in the finished sections may show up in a shade indistinguishable from that given by lipase. However, differential diagnosis is easy. Pigments are visible in unincubated sections, whereas lipase is not. The prussian blue reaction in a duplicate slide will eliminate the error due to hemosiderin, and the von Kossa silver stain, that due to the presence

of calcium salts. The latter can also be removed by treating the slide for ten to fifteen minutes with a citrate buffer of p_H 4.5 to 5.

RESULTS IN GENERAL

The activity of lipase, unlike that of the phosphatases, which give diffuse staining, is morphologically associated with cytoplasmic granules, which in some tissues may be very coarse (fig. 1). The nuclei are unstained. The pattern of distribution obtained in the same organ in different individuals of the same species is remarkably constant, although the intensity of the reaction may vary considerably. In different species the same organ may show different patterns. All substrates used gave identical pictures.

OBSERVATIONS ON NORMAL TISSUES AND ORGANS

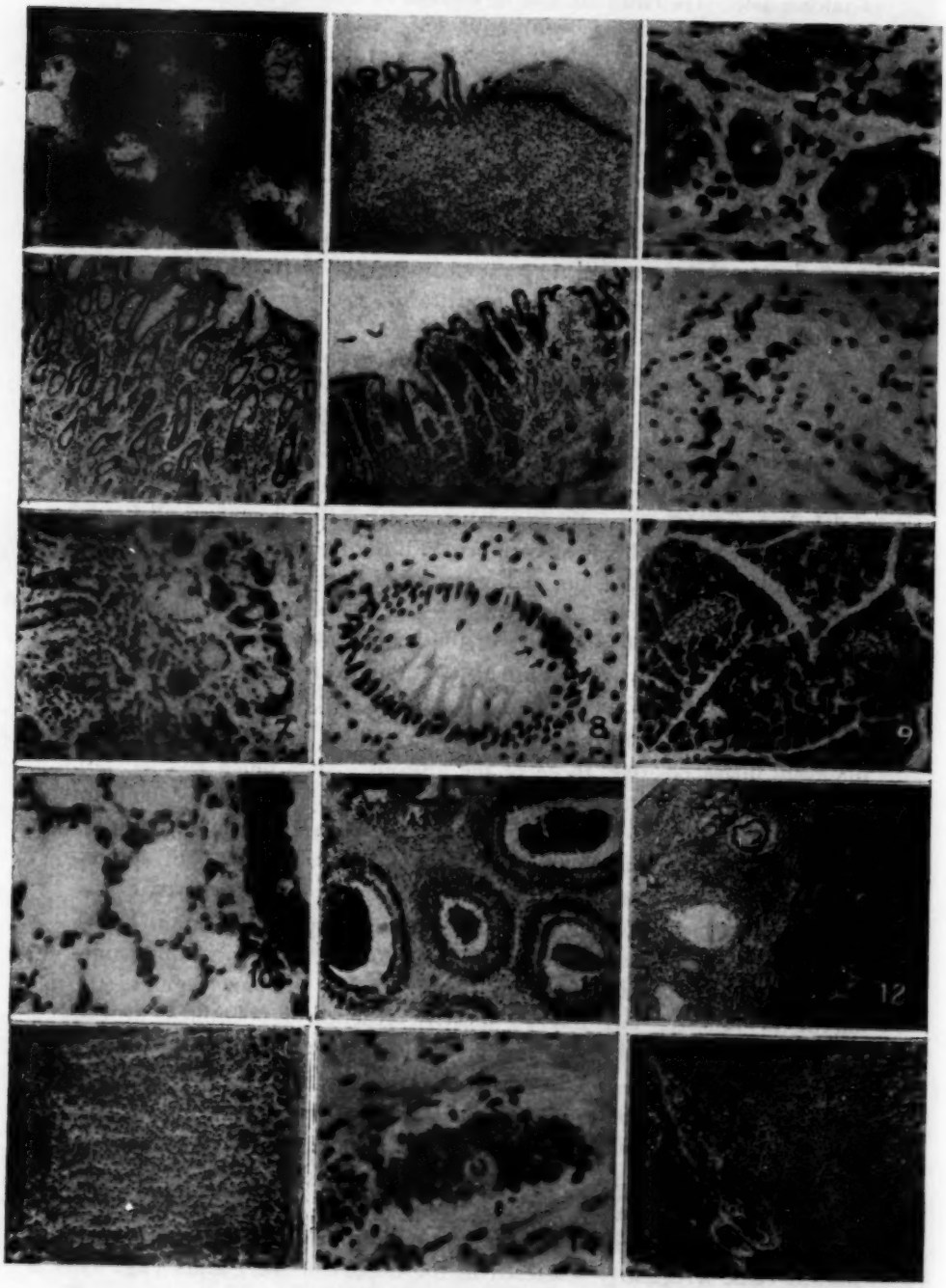
Vascular and Hemopoietic System.—In all species the blood vessels, as a rule, show no lipase activity. Occasionally, however, a positive reaction is obtained in the intima and the adventitia of medium-sized and large arteries, especially in pregnant rats. Blood cells of all kinds show no reaction. This is at variance with the findings of Barnes,[2] who was able to demonstrate minimal amounts of lipase in lymphocytes and leukocytes. The amount of enzyme present in these cells is apparently below the threshold of sensitivity of the histochemical method. Spleen, lymph nodes and bone marrow are also without evidence of lipase activity.

Digestive System.—The salivary glands of the guinea pig and the rat were examined. In the guinea pig the cells of the serous glands are intensely lipase positive, those of the mucous glands much less so. In the rat there are scattered cells outside the alveoli themselves, both in serous and in mucous glands, which contain positively staining granules. These cells are tentatively identified as basket cells. In both species the excretory ducts are entirely without evidence of this activity.

The deep layers of the epithelium of the esophagus contain lipase in all species examined. In animals the positive reaction is quite uniform, while in man it is patchy, being present in some regions and absent in others. An intense positive reaction is obtained in the esophagus in pig embryos of a crown-breach length of 11 cm. and above (fig. 2).

The distribution of lipase in the stomach varies greatly with different species. In the human stomach the fundic portion shows lipase in the zymogenic cells only, especially in the depths of the glands (fig. 3). The parietal cells are entirely without evidence of it. The antral and pyloric regions, together with the duodenum, show intensely stained cells, irregularly scattered in the depths of the glands (fig. 4).

2. Barnes, J. M.: Brit. J. Exper. Path. **21**:264 1940.



(See legends on opposite page)

With routine stains these lipase-containing cells are indistinguishable from the majority of those which show no reaction. In the dog the epithelium of the surface and of the necks of the glands shows lipase activity (fig. 5), much more in the fundic than in the antral portion. There is no reaction in the depths of the crypts. In addition, there are minute groups of small, obviously not epithelial cells present in the stratum proprium of the stomach, the duodenum and the small intestine showing an intense reaction (fig. 6). The nature of these cells is not clear. The duodenal and jejunal villi are without evidence of lipase activity. In the rabbit, the guinea pig and the rat the entire stomach appears free of lipase, but the epithelium of the duodenal and jejunal villi shows a positive reaction, with the exception of the goblet cells. In the rabbit, around the periphery of Brunner's glands (fig. 7) there are crescent-shaped groups of cells showing intense staining.

The colon and the appendix of man present a picture similar to that of the antrum of the stomach except for the fact that the lipase-containing cells are much more sparse (fig. 8). In all other species, the rhesus monkey excepted, the colon shows no lipase activity. In the rhesus monkey the entire epithelium of the colon, goblet cells included, is lipase active.

The liver presents an intensely positive reaction in all species examined. Whereas in most species the reaction is of a fairly uniform intensity throughout the lobules, in the rat the central areas are often much more intensely stained than the peripheral ones. The connective

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EXPLANATION OF PLATE.

Fig. 1.—Coarse lipase-positive granules in a rabbit's liver. × 266.5.

Fig. 2.—Cardia of an 11 cm. (crown-breech length) pig embryo. Deep layers of esophageal lining show intense lipase activity. × 66.5.

Fig. 3.—Fundic portion of a human stomach, showing a positive reaction in zymogenic cells and absence of reaction in parietal cells (arrows). × 266.5.

Fig. 4.—Antral portion of a human stomach. Note the scattered dark cells in the depths of the crypts. × 66.5.

Fig. 5.—Fundic portion of a dog's stomach showing a surface reaction. × 66.5.

Fig. 6.—Fundic portion of a dog's stomach. Scattered dark cells are seen in the stratum proprium. × 185.

Fig. 7.—Distal portion of a rabbit's duodenum. Crescent-shaped groups of cells show lipase activity. × 133.

Fig. 8.—Human appendix. Two cells showing lipase activity are shown by arrows. × 266.5.

Fig. 9.—Pancreas of a rat. × 66.5.

Fig. 10.—Lung of a rat. An intensely positive reaction is present in the bronchial epithelium and in the septal cells. × 266.5.

Fig. 11.—Epididymis of a dog. × 66.5.

Fig. 12.—Ovary of a dog, showing part of a corpus luteum. × 66.5.

Fig. 13.—Human adrenal gland containing in the zona reticularis a number of cells showing the specific staining. × 66.5.

Fig. 14.—A ganglion of Auerbach's plexus in a rhesus monkey. The ganglion cells show no lipase; the satellite cells present a positive result. × 266.5.

Fig. 15.—Adrenal gland of a rat with the periadrenal fat intensely stained. × 66.5.

tissue of the liver is entirely without evidence of lipase activity, and so are the Kupffer cells. In the rat the lining of the bile ducts reveals a positive reaction, but in all other species it shows little or no lipase activity.

In all species the pancreas shows intense staining of the zymogen granules (fig. 9) while the peripheral portion of the acinous cells may be unstained. The secretions within the ducts are also intensely reactive. The islets of Langerhans are nonreactive throughout. The addition of bile salts to the incubating mixture causes a considerable intensification of the lipase picture of the pancreas, while it reduces the intensity of the reaction in all other organs. This is in complete agreement with the findings of Willstätter and Memmen.[3]

Respiratory System.—The bronchial epithelium shows a positive reaction in all species examined, although in man in a patchy way. Some of the bronchial glands also are stained. The septal cells are intensely stained in a uniform and selective way in the rat (fig. 10) and in the guinea pig, less intensely in the rabbit and inconstantly in the dog. In man, the attached septal cells are without lipase activity but the desquamated ones show a positive reaction.

Uropoietic System.—In man the kidney is entirely without lipase activity. In the dog the cells of the convoluted tubules stain very weakly, but the straight portion of the proximal tubules down to the transition into Henle's loop shows more or less intense activity of lipase. The tubules distal to this transition point do not show lipase activity. The epithelium of the renal pelvis and of the urinary bladder presents a positive result. In the guinea pig the entire kidney, as a rule, is free from lipase activity, although occasionally the convoluted tubules may be slightly stained. In the rabbit and the rat, both the proximal and the distal convoluted tubules show lipase activity, while Henle's loops and the collective tubules are without evidence of it. This is in agreement with the data of Weil and Jennings.[4] The epithelium of the renal pelvis and of the urinary bladder shows a positive reaction in the guinea pig and the rabbit and no reaction in the rat.

Male Genital System.—In man, the seminiferous tubules show no lipase activity, while the interstitial cells show a positive reaction. In the rabbit and the rat the picture is very similar. In the dog the Sertoli cells contain coarse lipase-positive granules; the interstitial cells contain none. The testis of the guinea pig is entirely without evidence of lipase activity. The epithelium of the epididymis shows a positive result to a varying extent in all species examined; the brush border may show especially heavy staining (fig. 11). In the guinea pig,

3. Willstätter, R., and Memmen, F.: Ztschr. f. physiol. Chem. **138**:216, 1924.
4. Weil, L., and Jennings, R. K.: J. Biol. Chem. **139**:421, 1941.

the epithelium of only the distal portion of the epididymis shows staining, while that of the proximal portion is entirely free from evidence of lipase activity. There is an interesting reciprocal relationship between lipase and alkaline phosphatase pictures in that the basement membrane of the lipase-containing distal part shows no phosphatase reaction, whereas that of the lipase-free proximal portion shows intense phosphatase activity.

The prostate in man reveals no evidence of lipase activity. In the dog the lining of the excretory ducts and of some of the glands shows moderate staining.

Female Genital System.—In woman, the ovary and the nonpregnant uterus are without lipase; the decidua of the pregnant uterus shows a patchy lipase reaction. In the dog, the rabbit, the guinea pig and the rat, the cells of the corpora lutea contain very coarse lipase-positive granules. Especially intense is the reaction in the dog's ovary (fig. 12). In addition, an intense reaction was found in the theca of ripening follicles in 1 pregnant rat. The uterus and the tubes are without evidence of lipase activity in all animal species.

Endocrine Glands.—The adrenal gland shows a marked species difference in the lipase picture. In man a varying number of cells in the zona reticularis are lipase active (fig. 13). In the dog the entire zona glomerulosa and irregular groups of cells in the zona reticularis show the reaction. The guinea pig's adrenal gland appears entirely free of lipase. In the rabbit there is an inconstant patchy reaction of moderate intensity extending from the zona glomerulosa into the zona fasciculata. The rat's adrenal gland is either entirely without lipase activity or shows a very faint reaction in the zona glomerulosa and the outer portion of the fasciculata. The medulla in all species reveals no lipase.

The thyroid gland, the parathyroid gland and the thymus show no lipase in any species. The pituitary gland was not examined.

Nervous System.—Tissues of the nervous system were not examined. The only positive finding was a distinct reaction in the satellite cells of the Auerbach plexus in the rhesus monkey (fig. 14) and a much less conspicuous but similar reaction in the rabbit.

Muscles, Skin and Its Appendages.—These organs are without evidence of lipase activity in all species.

Connective Tissue.—Groups of brown-staining fibroblasts are occasionally seen in various organs of all species. Especially in embryos, strands of intensely stained fibroblasts are seen in the walls of the gastrointestinal tract. The adipose tissue shows a consistently positive result in the rabbit and in the rat (fig. 15) and a negative result in the other species, although groups of moderately stained fat cells are sometimes observed in human adipose tissue.

OBSERVATIONS ON PATHOLOGIC TISSUES AND ORGANS

Miscellaneous Non-Neoplastic Changes (Inflammatory and Degenerative Processes; Granuloma).—Inflammatory processes do not change the lipase picture unless they lead to necrosis. Necrotic tissue, regardless of its origin, contains no or very little lipase. No lipase is found in fatty degeneration of the kidney or of the myocardium. Fatty livers contain less lipase than normal ones. On the other hand, in atheromatous patches of the aorta many of the fat-laden macrophages contain the brown-staining granules (fig. 16). The foam cells in 2 cases of Gaucher's disease were entirely without lipase activity.

In cirrhosis of the liver, the regenerated, large liver cells are more intensely stained than the rest of the lobule.

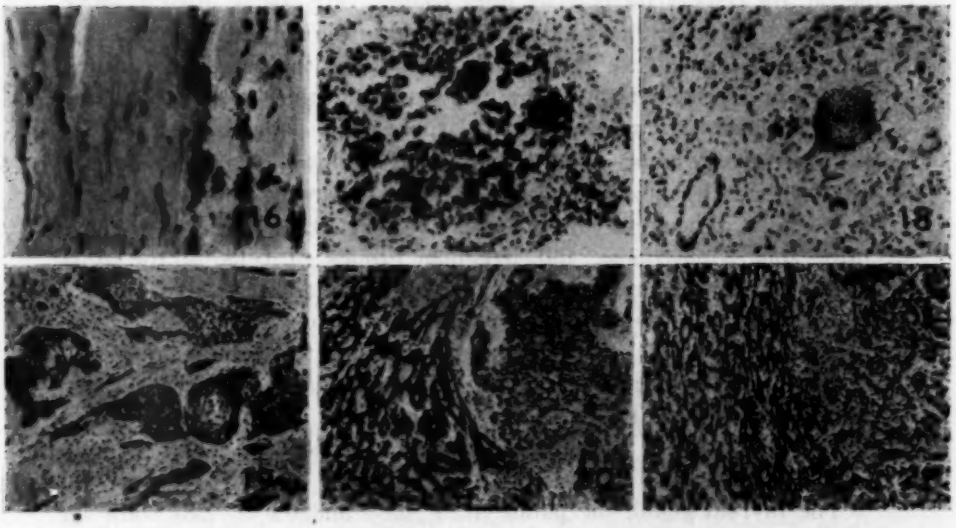

Fig. 16.—Atheromatous patch of a human aorta with lipase active fat-laden macrophages. × 266.5.

Fig. 17.—Tubercle in a rabbit's lung. The epithelioid and the giant cells show a positive result. × 133.

Fig. 18.—Tubercle in a human kidney. A giant cell full of coarse lipase-active granules is seen. × 133.

Fig. 19.—Squamous carcinoma of the esophagus. Most of the tumor cell nests show a positive reaction. × 66.5.

Fig. 20.—Lipase-free hepatic metastasis of gastric carcinoma. The liver tissue itself shows positive staining. × 66.5.

Fig. 21.—Hepatoma. Both the tumor and the liver tissue show a positive reaction. × 66.5.

In tuberculosis, both the epithelioid and the giant cells are rich in lipase-positive granules, especially around the periphery of the tubercle. The reaction is intense and widespread in the rabbit (fig. 17), less intense and patchy in distribution in man (fig. 18). The necrotic

centers of tubercles contain no lipase. A picture similar to that seen in tuberculosis but of a lesser intensity is observed in sarcoidosis.

Tumors.—A large number of specimens of carcinoma and sarcoma of various organs were examined. All of them were found to be without lipase activity (fig. 19) except for 1 specimen of squamous carcinoma of the esophagus (fig. 20), 1 of hepatoma (fig. 21) and 1 somewhat atypical specimen of seminoma. The first and second specimens stained fairly uniformly, while in the third the seminoma cells themselves were without evidence of lipase activity but the stroma contained a large number of scattered spindle-shaped and polyhedral cells, the nature of which could not be established, which showed presence of lipase.

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

XII. Atheromatosis in Dogs Following Repeated Intravenous Injections of Solutions of Hydroxyethylcellulose

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IN a series of experimental studies[1] it was shown that the prolonged parenteral introduction of large amounts of colloidal solutions of macromolecular carbohydrates (polyvinyl alcohol, methyl cellulose, pectin, sodium cellulose glycollate, acacia) elicits in the arteries of dogs and rabbits atheromatous lesions which contain the injected foreign matter in foam cells. When, therefore, hydroxyethylcellulose (Cellosize, produced by the Carbide and Carbon Chemical Corporation) became available, it appeared to be of interest to test this substance for any potential atheromatogenic properties.

EXPERIMENTAL PROCEDURE

Hydroxyethylcellulose is a white powder which is manufactured in three grades of viscosity: (WS-20 having a viscosity of 11.3 centipoises at 20 C. as a 5 per cent aqueous solution, WS-100 having a viscosity of 68.7 centipoises under these conditions and WS-500 having a viscosity of 465 centipoises). The reactions of such solutions are slightly below the neutral point (p_H 6.6 to 8.6). The viscosity of each of these solutions decreases with increasing temperature. The compound is more soluble in hot water than in cold. The solutions do not gel at higher temperatures as do solutions of methyl cellulose. The aqueous solutions are translucent and slightly turbid. When given orally to rats, the substance is less toxic than glycerin, according to the statement of the manufacturer. It does not exert any irritating effect when applied to the skin but has a mild irritating action on the conjunctiva of the rabbit.

For the purpose of the experiment, the three types of hydroxyethylcellulose were prepared in isotonic solution of sodium chloride. In these solutions the concentration of hydroxyethylcellulose was adjusted in such a way that their viscosity was five to six times that of normal plasma or ten to twelve times that of water. The solutions used had the following concentrations: High viscosity grade, 2.3 per cent; medium viscosity grade, 5.0 per cent, and low viscosity grade, 10.0 per cent. They were injected into the jugular veins of dogs in amounts of 50 cc. daily after being filtered, while hot, through filter paper in a Buechner filter. Two dogs were used for the testing of each of the three

From the Warner Institute for Therapeutic Research.

1. (*a*) Hueper, W. C.: Arch. Path. **28**:510, 1939; (*b*) **31**:11, 1941; (*c*) **33**:1, 1942; (*d*) Am. J. Path. **20**:737, 1944; (*e*) Arch. Path. **34**:883, 1942; (*f*) Experimental Studies in Cardiovascular Pathology: XIV. Experimental Atheromatosis in Macacus Rhesus Monkeys, Am. J. Path., to be published; (*g*) ibid. **18**:895, 1942; (*h*) Arch. Path. **33**:267, 1942. (*i*) Hueper, W. C.; Landsberg, J. W., and Eskridge, L. C.: J. Pharmacol. & Exper. Therap. **70**:201, 1940.

types of solutions. The dogs ranged in weight from 7.5 to 12.9 Kg. and in age from 10 months to 3 years, estimated from the condition of their teeth at the start of the experiment. The animals were killed at the end of the experimental period by intravenous injection of a 10 per cent formaldehyde solution.

The dogs remained in good health during the experiment. There were no major fluctuations in weight. The injections were in general well tolerated. Vomiting was observed only a few times, occurring in each instance shortly after the injection. There were brief moderate rises in temperature on several occasions in spite of the fact that the solutions were prepared with pyrogen-free water.

Studies of blood were made directly before, and five, ten, thirty minutes, two, five and twenty-four hours after, the first injection and at weekly intervals thereafter when injections were made five times a week over periods of six to twelve weeks. They included erythrocyte and leukocyte counts, a differential count of leukocytes, determinations of hemoglobin, coagulation time, sedimentation rate, volume of packed blood cells and viscosity of plasma.

HEMATIC OBSERVATIONS

Hydroxyethylcellulose WS-500 (high viscosity grade).—The 2 dogs which received injections of a 2.3 per cent solution of the highly viscous hydroxyethylcellulose for periods of six and seven weeks, respectively, exhibited the following acute hematic reactions during the twenty-four hour period after the first injection of 50 cc. of this material. The number of erythrocytes and the amount of hemoglobin underwent only minor and inconsistent fluctuations. The number of leukocytes dropped at the five minute test to about half of the original number and remained at this level for approximately two hours. The original level was again attained at the five hour test. There was a moderate decrease (33 per cent) in the number of the polymorphonuclear leukocytes during the leukopenic phase, which was associated with relative lymphocytosis. The fluctuations in coagulation time and volume of packed blood cells were insignificant. The erythrocytic sedimentation rate was markedly increased in the five minute test and remained high (peak 51 mm. with Wintrobe-Landsberg tube) in one animal up to the twenty-four hour test, while it returned to normal values in the twenty-four hour test in the other. The viscosity of the plasma increased in the five minute sample from an original value of 1.6 and 1.9, respectively, to 2.7 centipoises. At the five hour test the value was 2.35, and it remained at this level at the twenty-four hour test.

During the chronic phase of the experiment there were no appreciable changes in the number and the ratio of leukocytes and in the coagulation time. The number of erythrocytes, the amount of hemoglobin and the volume of packed blood cells dropped progressively until they reached at the end of the experimental period about one half of the original values. The sedimentation rate was consistently and considerably increased except at times when in one of the dogs the plasma viscosity reached values above 5 and the sedimentation rate decreased sharply. No such relation was found in the second dog. The viscosity of the plasma was definitely and increasingly elevated, reaching at times values of 6 and a peak value of 8.

Hydroxyethylcellulose WS-100 (medium viscosity grade).—Two dogs received intravenous injections of 50 cc. of a 5 per cent solution of the medium viscous hydroxyethylcellulose five times weekly for a period of eleven weeks. The following acute hematic reactions were noted during the first twenty-four hours following the initial injection. At the ten minute test there was a drop in the

number of erythrocytes amounting to more than 2,000,000 cells in both dogs. This hemodilution remained in effect, though to a lessened degree, for over five hours, with almost original values prevailing at the twenty-four hour test. There was a corresponding reduction in the amount of hemoglobin during this period. The volume of packed red cells was paradoxically increased during the erythropenic period and reached especially high values when the reduction of the number of red cells was most severe. A marked colloidoclastic leukopenic response appeared at the five minute test, at which the number of leukocytes was reduced to about one third of the original number. This reaction persisted for thirty to sixty minutes, after which the number of leukocytes returned to an approximately original level. There were no considerable shifts in the ratio of the various types of leukocytes during this leukopenic response except in 1 dog at the five minute test, when lymphocytosis existed. The coagulation time remained unchanged. The sedimentation rate was only moderately increased. The viscosity of the plasma rose from 2.1 and 2.2, respectively, to 2.75 and 3.0, respectively, at the five minute test, and remained between 2.7 and 2.3 for the next five hours, being in one of the dogs still elevated in the twenty-four hour test (2.5).

Repeated intravenous injections of solutions of the medium viscous hydroxyethylcellulose caused a moderate drop in the number of erythrocytes, the amount of hemoglobin and the volume of packed blood cells. It was found on several occasions that the volume of packed blood cells was abnormally high (up to 74 mm.) despite a definitely reduced number of erythrocytes (3,660,000). The sedimentation rate was moderately to considerably accelerated.

Hydroxyethylcellulose WS-20 (low viscosity grade).—Two dogs received intravenous injections of a 10 per cent solution of the low viscous hydroxyethylcellulose for twelve weeks five times a week. One dog exhibited during the first twenty-four hours after the first injection a mild to moderate decline in the number of erythrocytes and in the amount of hemoglobin. There occurred, on the other hand, a marked increase in the volume of packed blood cells of this dog at the five and twenty-four hour tests, when this value stood at 71 and 73 mm., respectively, despite a slightly to moderately lowered number of red cells. No such reactions were found in the second dog. The number of leukocytes fell sharply in both dogs at the five minute test to one third and to one fourth, respectively, of the original value and remained reduced for ten minutes to two hours. This reaction was accompanied by a considerable increase in the number of lymphocytes and a corresponding drop in the number of neutrophilic leukocytes at several but not all tests showing a leukopenic response. The coagulation times of both dogs and the sedimentation rate of one dog remained unchanged. There was a moderate acceleration of the erythrocytic sedimentation in the second dog lasting for two hours. The plasma viscosity was mildly elevated directly after injection, but this fluctuation stayed within normal limits.

After repeated intravenous administrations of this material there developed toward the end of the experimental period a moderate degree of anemia. Again it was noticed that the values of the volume of packed blood cells were at times abnormally high in comparison with the lowered number of erythrocytes. But this discrepancy was not as marked and as frequent as in the previous series. The number of leukocytes and their ratio fluctuated within normal limits during the experimental period. There were no abnormal changes in the coagulation time. The sedimentation rate was mildly, but not always, increased. The viscosity of the plasma remained within the normal range in one dog but was elevated in the other dog, rising to a peak value of 3.25.

ANATOMIC OBSERVATIONS

The postmortem examination of the 6 dogs did not reveal any considerable deviation from normal conditions with the exception of those of the spleens, which were mildly to moderately increased in size, pinkish red and medium firm in consistency. The livers were normal in size and appearance.

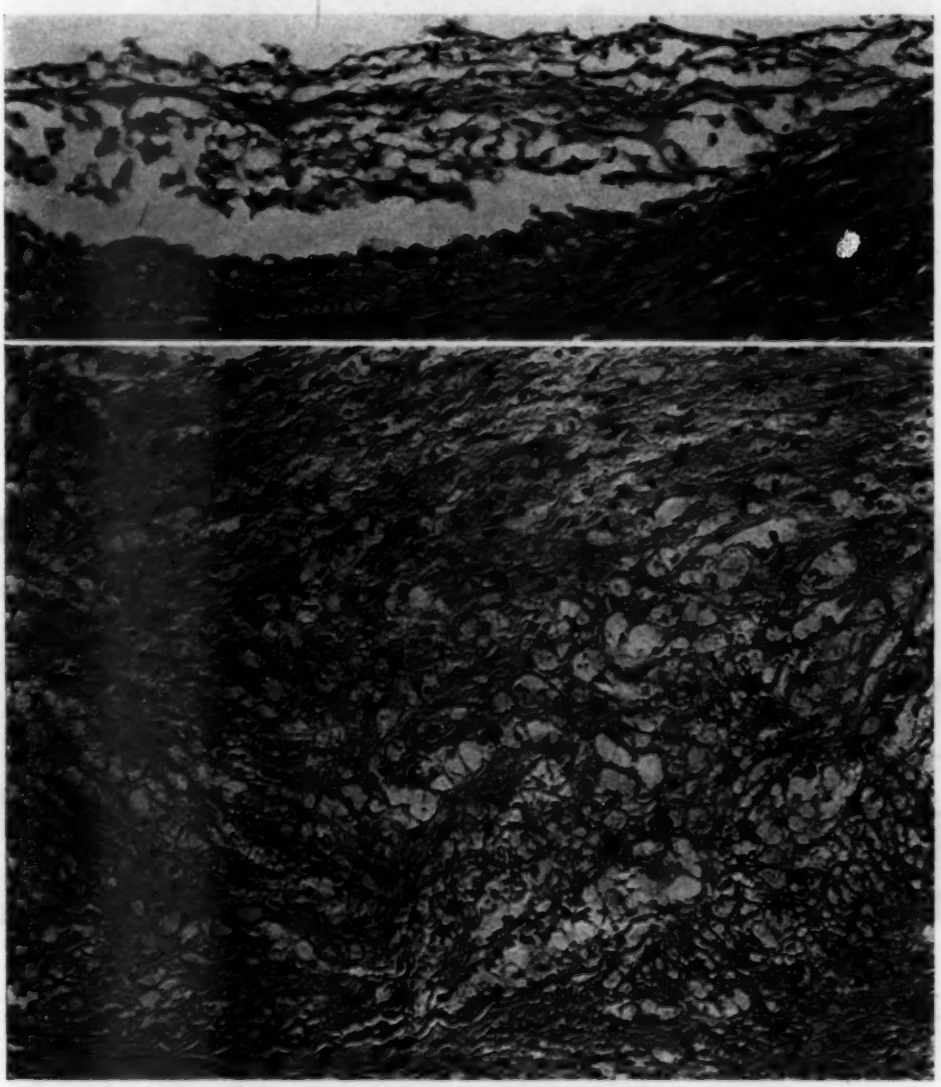

Fig. 1.—Thickened intima of an epicardial coronary artery, composed of foam cells and fibroblasts.

Fig. 2.—Large intimal cushion of the abdominal portion of the aorta, consisting mainly of fibrous tissue, with foam cells accumulated at its base.

A histologic examination was made of the following organs: thyroid gland, parathyroid glands, lungs, heart, aorta, carotid arteries, iliac arteries, brachial arteries, vena cava inferior, liver, spleen, pancreas, adrenal glands, kidneys, testis, prostate and bone marrow.

The organs of the 2 dogs which received the highly viscous hydroxyethylcellulose were essentially without any abnormal changes. The spleen of each showed a moderate increase of the reticular cells. The intima of the bulb of

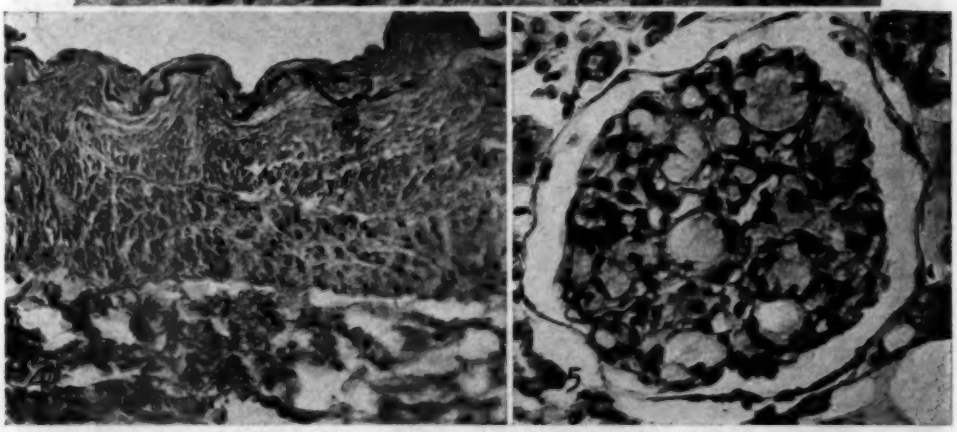

Fig. 3.—Foam cell proliferation of the endothelium of a medium-sized artery, forming a thick cushion.

Fig. 4.—Hyalinization and calcification of the media involving the elastica of a medium-sized artery.

Fig. 5.—Glomerulus with cystically dilated capillary loops containing homogeneous matter.

the aorta was locally thickened and consisted there of an edematous cushion of hydropic cells. There were no atheromatous lesions or other changes in the aorta and the arteries studied. The liver cells exhibited coarsely flaky cytoplasm.

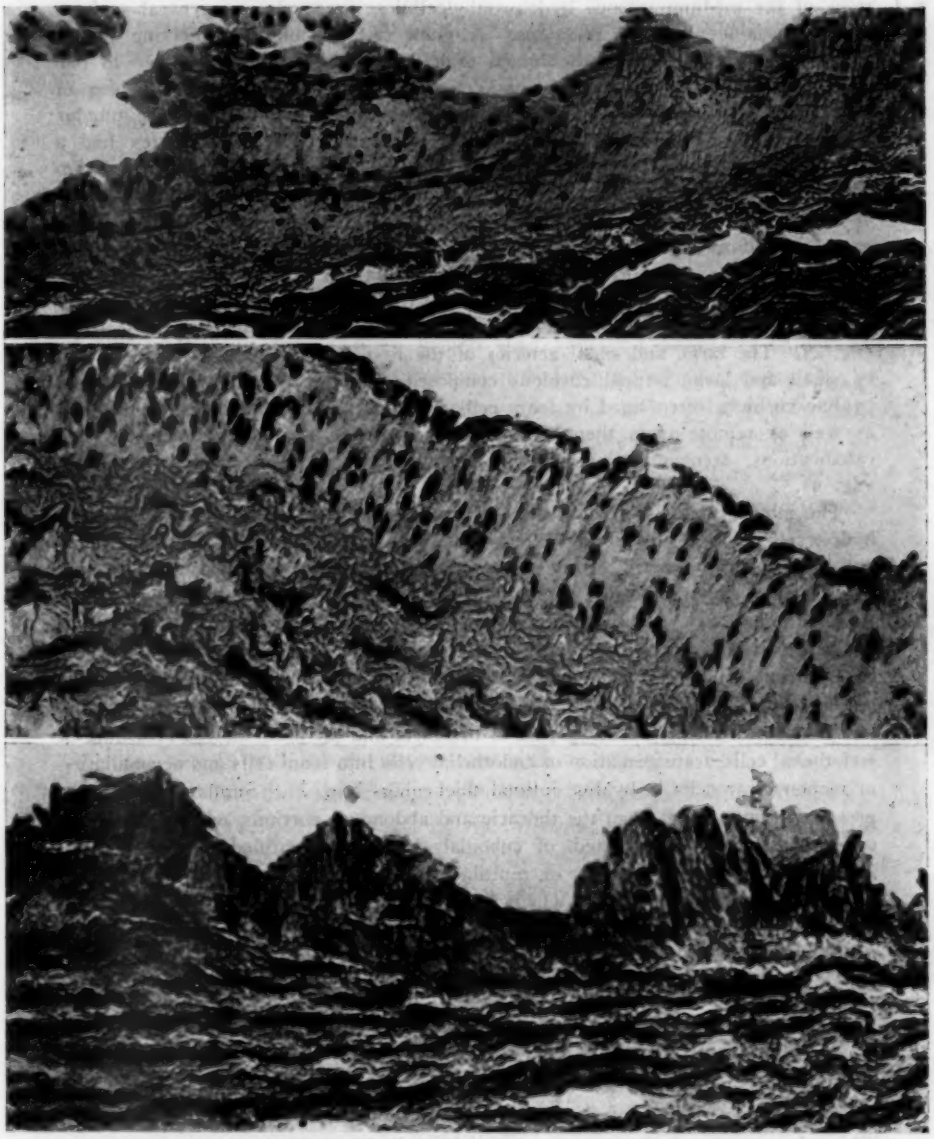

Fig. 6.—Foam cell proliferation of the endothelial lining of the left ventricle.

Fig. 7.—Crowding of endothelial cells and hyaline thickening of the intima, which contains mononuclear cells, of the ascending part of the aorta.

Fig. 8.—High cuboidal and cylindric endothelial cells forming small tonguelike cushions in the thoracic and abdominal portions of the aorta.

A few small interstitial round cell infiltrations were present in the kidneys of one dog and small calcium casts in the medullary tubular lumens of the kidneys of the other.

The parenchymatous organs of the 2 dogs which received injections of solutions of the medium viscous hydroxyethylcellulose were also in general without any appreciable abnormal reactions. A giant cell granuloma projecting like a broad-based papilloma into the lumen of a pulmonary vessel was noted in one dog. The cytoplasm of the hepatic cells was coarsely flaky. The spleen of each animal showed an increase of the reticular cells. In both dogs the tubular lumens in the renal medulla contained calcium casts. The renal cortex had a few interstitial round cell infiltrations in one dog. An appreciable accumulation of foam cells was found beneath the endothelium of a pericardial coronary artery of this dog (fig. 1). In both dogs the aortas revealed in the bulbar portion narrow cushions of hydropic tissue in which mononuclear cells were embedded. The upper end of the aortic bulb of one dog contained a large calcified cartilaginous focus in the media. The abdominal portion of the aorta of the second dog showed large fibrous intimal thickenings with scattered foam cells at their bases (fig. 2). The large and small arteries of the first dog were extensively effected by small and large intimal cushions composed of foam cells and of fibrous and hyaline cushions often lined by foam cells (fig. 3). The media beneath such foci as well as remote from them revealed circumscribed medial hyalinizations and calcifications, frequently involving fragmented and swollen elastic membranes (fig. 4).

The internal organs of the 2 dogs which received injections of the low viscous hydroxyethylcellulose exhibited extensive lesions. The liver cells were distended and had sharply defined cellular membranes and foamy cytoplasmic structure. The splenic reticular cells were distinctly proliferated. The renal glomeruli revealed numerous cystically dilated capillary loops containing either a homogeneous grayish matter or endothelial foam cells (fig. 5). The endothelial cells lining the left ventricle of one dog were markedly swollen and cuboidal or columnar in shape, were arranged in several layers and, sometimes, surrounded hyaline material (fig. 6). In both dogs the ascending part of the aorta and the arch showed crowding of endothelial cells, transformation of endothelial cells into foam cells and accumulation of mononuclear cells in hyaline intimal thickenings (fig. 7). Similar lesions were present in rings taken from the thoracic and abdominal portions, where endothelial cells were locally proliferated, of cuboidal shape and stratified in arrangement, forming tonguelike projections or multilayered coatings (fig. 8). In some parts actual cushions had developed, which were composed of foam cells. In both dogs the outer media of one of the large aortic rings showed extensive hyalinizations of the outer and middle portions of the media, which were infiltrated with small round cells. A small artery was completely lined by a thick coat of foam cells.

COMMENT

The most pronounced and most prolonged leukopenia followed the intravenous injection of the most viscous type of hydroxyethylcellulose. This observation is in agreement with a similar one made in connection with the testing of 7 types of methyl cellulose of graduated molecular weights and viscosity.[1d] Corresponding relationships were found concerning the degree and the duration of the acceleration of erythrocytic sedimentation, the relative increase in the viscosity of the plasma and

the degree of anemia which developed after single and repeated injections of these substances. It was noted, on the other hand, that the medium viscous hydroxyethylcellulose exerted the most pronounced hemodiluting action; such action was of only moderate degree with the low viscous type and entirely absent with the highly viscous variety. It is doubtful whether or not such a hydrating effect is responsible for the discrepancy repeatedly observed between the number of erythrocytes and the volume of packed blood cells, which was found at several occasions to be abnormally high during the chronic part of the experiment. It is possible that a swelling of the erythrocytes may have been responsible for such a phenomenon. As the average cell size was not determined, this explanation must remain a tentative one.

The histologic observations were to a certain extent unexpected. The practically complete absence of any significant abnormal changes in the organs of the 2 dogs which had received repeated injections of the highly viscous preparation was noted with surprise, especially as these animals had shown the most pronounced and prolonged hematic reactions, indicating that considerable amounts of the injected foreign colloid were present in the blood. It may be recalled in this connection that the dogs which received injections of the two methyl celluloses of highest viscosity exhibited in the internal organs and the arterial vessels anatomic lesions of a lesser degree than those observed in dogs treated with methyl celluloses of lower viscosity. It is not thought that the difference in the absolute amounts of the various hydroxyethylcellulose injected and in the durations of the treatment entirely accounts for these discrepancies, as the more viscous and, therefore, higher macromolecular hydroxyethylcellulose should have been retained more readily than the less viscous and lower macromolecular varieties.

It was found that the 2 dogs which received injections of hydroxyethylcellulose WS-20 exhibited in the internal organs and the arteries the most severe and generalized lesions of the three groups studied. Only the animals of this series showed phenomena related to the storage of the injected matter by the presence of swollen liver cells with foamy cytoplasm and by the accumulation of grayish-stained homogeneous matter in the lumen and endothelial lining of the capillary loops of the renal glomeruli. No such reactions were seen in the dogs of the other series. Foam cells and fibrous cushions and circumscribed hyaline thickenings of the intima, often associated with degenerations and calcifications of the media of the aorta and of large and medium-sized arteries, were seen in the dogs which received injections of hydroxyethylcelluloses of medium and low viscosity. These lesions were definitely more extensive and more widely distributed in the dogs which received the low viscous preparation than in those treated with the medium viscous one. In the former group, moreover, swelling

and proliferation of the endocardial cells were present. It is noteworthy that also this atheromatogenic agent elicited both atheromatous intimal and degenerative and calcifying medial reactions such as those observed after the injection of methyl cellulose. The predominantly fibrous character of some of these lesions indicates a high degree of lability of the foam cell reactions elicited by hydroxyethylcellulose and thus presents an analogy to the early fibrous transformations of the atheromatous changes seen after the injection of pectin.

SUMMARY

Hydroxyethylcellulose of three grades of viscosity dissolved in isotonic solution of sodium chloride was injected intravenously into dogs without eliciting any acute and serious untoward reactions.

The most viscous hydroxyethylcellulose caused the most pronounced symptoms (leukopenia, accelerated erythrocytic sedimentation, anemia) characteristic of the macromolecular hematologic symptom complex.

The medium viscous hydroxyethylcellulose produced the most pronounced hemodiluting action.

Storage phenomena in the liver cells and the glomerular endothelial cells, as well as atheromatous and fibrous intimal lesions and medial degenerations and calcifications, were most extensive and most widely spread in the dogs which received injections of solutions of the low viscosity material. Such reactions were entirely absent in the dogs treated with the high viscosity preparation.

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

XIII. Vibratory Lability of Plasma Colloids in Rabbits and in Dogs Following Ingestion of Cholesterol

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IN PREVIOUS publications [1] the theory was advanced that local vibratory influences acting on the blood may control to a certain extent the lability of the plasma colloids. This effect in the presence of quantitative disturbances of the cholesterol content of the plasma or of qualitative disturbances of its physicochemical status may cause precipitation of cholesterol at certain parts of the vascular tree where the vibratory factors are most active. This mechanism is believed also to account for the local distribution of atheromatous lesions, which are found at the orifices of the aortic branches, at natural and abnormal narrowings, sacculations and curves of arteries and at areas of normal or pathologic whirl formation and pulsatory impacts of the blood, such as the ascending part of the aorta and the first portion of the coronary arteries.

The studies to be reported were undertaken to obtain additional support for this theory. The vibratory lability of the serum of normal and cholesterol-fed rabbits and dogs was investigated with and without the admixture of various detergents, macromolecular colloidal substances and other agents which exert an influence on the colloidal stability of plasma or which had been successfully used in preventing the development of experimental cholesterol atheromatosis in rabbits.

EXPERIMENTAL PROCEDURES AND OBSERVATIONS

Normal Rabbit Serum Plus Macromolecular Substances.—It was shown in experiments reported during recent years [2] that in dogs and rabbits repeated and prolonged intravenous injections of large amounts of solutions of various hydrophilic carbohydratic colloids (polyvinyl alcohol, methyl cellulose, pectin, sodium cellulose glycolate, hydroxyethylcellulose, acacia) and proteinic colloids (gelatin, ovalbumin) result in specific intimal lesions of foam cell atheromatous or fibrohyaline character, respectively, of the arteries. It was therefore of interest to determine the behavior of these substances when they were mixed with serum

From the Warner Institute for Therapeutic Research.

1. Hueper, W. C.: (*a*) Arch. Path. **38**:162, 245 and 350, 1944; **39**:51, 117 and 187, 1945; (*b*) this issue, p. 130; (*c*) Experimental Studies in Cardiovascular Pathology: IV. Experimental Atheromatosis in Macacus Rhesus Monkeys, Am. J. Path., to be published.

2. Hueper, W. C.: (*a*) Arch. Path. **31**:11, 1941; **33**:1, 1942; **34**:883, 1942; (*b*) this issue, p. 130; (*c*) Am. J. Path. **18**:895, 1942; **20**:737, 1944.

and the mixture exposed to prolonged vibration. For the purpose of the experiment 1 part of a 0.5 per cent solution of pectin in isotonic solution of sodium chloride was mixed with 2, 5, 10, 20, 50, 100 and 200 parts of normal rabbit serum; 2 cc. quantities of these mixtures were then placed in test tubes such as are generally used for the Kahn test, incubated for one hour at 37 C. and then shaken for a period of thirty minutes in a small electric shaker such as is employed for the shaking of blood pipets. The tubes were then examined for floccules.

A similar arrangement was employed in examining the behavior of a 1.5 per cent solution of polyvinyl alcohol, a 5.0 per cent solution of acacia, a 0.25 per cent solution of methyl cellulose (viscosity, 400 centipoises), a 0.075 per cent solution of methyl cellulose (viscosity, 4,000 centipoises) and a 1.5 per cent solution of gelatin. All solutions used had the same viscosity as normal plasma.[2] The results obtained are given in table 1.

TABLE 1.—*Vibratory Lability of Mixtures of Rabbit Serum and Macromolecular Substances*

Substance	Mixture	Serum Control	Flocculation Observed in Mixture with Given Ratio of Macromolecular Solution to Serum							
			1:0	1:2	1:5	1:10	1:20	1:50	1:100	1:200
Pectin.....	S	0	0	0	Trace	0	0	0	0	0
	U	0	0	0	0	0	0	0	0	0
Polyvinyl alcohol....	S	0	0	+	+	+	+	+	+	+
	U	0	0	0	0	0	0	0	0	0
Acacia.....	S	0	0	+	+	0	0	0	0	0
	U	0	0	0	0	0	0	0	0	0
Methyl cellulose, 400 centipoises	S	0	0	0	0	0	0	0	0	Trace
	U	0	0	0	0	0	0	0	0	0
Methyl cellulose, 4,000 centipoises	S	0	0	0	0	0	0	0	0	0
	U	0	0	0	0	0	0	0	0	0
Gelatin.....	S	0	Trace	+	+	+	+	+	+	+
	U	0	0	0	0	0	0	0	0	0

S = shaken mixture; U = unshaken control.

Mixtures of solutions of polyvinyl alcohol and serum and of gelatin and serum exhibit under the experimental conditions used marked vibratory lability, evidenced by the appearance of floccules; of the mixtures of serum and solutions of acacia, only those with the higher concentrations of acacia display this quality and these to only a moderate degree; mixtures of serum and solutions of methyl cellulose or solutions of pectin do not form any precipitates, or only minor ones, when shaken.

Normal Rabbit Serum plus Detergents or Iodides.—In a second set of experiments the influence of small amounts of detergents and of organic iodides on the vibratory lability of normal serum was tested. The study of these two groups of agents was suggested by the fact that detergents generally increase the dispersion of fatty material in a watery medium and thus accentuate the stability of colloidal emulsions of this type, while iodides have been successfully used in the prevention of experimental cholesterol atheromatosis in rabbits. These agents were used as a 0.1 per cent watery solution and were added to the serum in these proportions: 5, 4, 3, 2, 1 and 0 parts making a total of 5 parts or 2 cc. The two liquids were thoroughly mixed and then shaken for thirty minutes.

The following substances were employed: of detergents, Tergitol Penetrant 08,[3] Tergitol Penetrant 4T, Tergitol Penetrant 7, Tergitol Penetrant 4, dehydrocholic acid, lecithin, Triton K-60,[4] Triton NE, Aerosol OT,[5] cetylpyridinium iodide and sodium hexametaphosphate; of iodides, beta iodopropionic acid, 3,5-*l*-diiodotyrosine, *n*-hexyl iodide and hexamethylenetetramine alliodide. The total amount of the mixture of serum and agent was always 2 cc. An unshaken tube having the same content served as control.

Cetylpyridinium iodide produced a precipitate in both the shaken and the unshaken tube. Sodium hexametaphosphate and lecithin had no demonstrable effect on the colloidal stability of the serum. When Targitol Penetrants 4, 4T and 7 were used, flocculation occurred in all the tubes, and the flocculation decreased in intensity with the decrease of the concentration of the Tergitols in the mixture. With Tergitol 08 there was flocculation in only the three highest concentrations, while with Aerosol OT this effect was observed in the two highest concentrations. Dehydrocholic acid elicited mild flocculation in the second and third highest concentrations. The unshaken tubes containing these mixtures remained clear. The effect of the iodides tested was in general negative with the exception of the appearance of floccules in the highest concentrations of both 3,5-*l*-diiodotyrosine and *n*-hexyl iodide. The readings in some of the tests on iodides was interfered with by the fact that flocculation appeared in the tubes containing untreated rabbit serum after shaking, indicating that in the blood of the normal rabbit substances occur at times which lower the colloidal stability of the serum in the presence of vibratory influences. It was noted, however, that in these instances there was no flocculation in the tubes containing the iodides in low concentration.

Normal Rabbit Serum Plus Detergents Plus Macromolecular Substances.—The observations made in the two experiments recorded suggested a test of the influence which a combination of serum, macromolecular substances and detergents might have on the vibratory lability of such mixtures. For this purpose normal rabbit serum was mixed with solutions of polyvinyl alcohol (1.5 per cent), pectin (0.5 per cent), methyl cellulose of a viscosity of 400 centipoises (0.25 per cent) and methyl cellulose of a viscosity of 4,000 centipoises (0.075 per cent) in a ratio of 1 part of macromolecular colloidal solution to 2, 5, 10 and 20 parts of serum. To 2 cc. of each mixture, 0.1 cc. of a 0.1 per cent watery solution of one or another of various detergents was added. The resulting mixture was shaken for thirty minutes. There were serum controls and macromolecular substance controls, as well as a set of unshaken tubes containing the same materials as were present in the shaken ones. The detergents used were Tergitol Penetrants 4, 4T, 7 and 08, Tritons NE and K-60, Roccal,[6] Aerosol OT, cetylpyridinium iodide, dehydrocholic acid and lecithin.

Tergitol 4, 4T, 7 and 08, Triton K-60, Triton NE, Roccal, Aerosol OT, cetylpyridinium iodide, lecithin and dehydrocholic acid did not elicit any flocculation in serum mixed with methyl cellulose of two viscosities (400 and 4,000 centipoises) and

3. Tergitol Penetrant 08 is sodium sulfate of 2-ethyl hexanol; Tergitol Penetrant 4, sodium sulfate of 2-ethyl hexanol; Tergitol Penetrant 4T, triethanolamine tetradecyl sulfate; Tergitol Penetrant 7, sodium alcohol sulfates 3,9-diethyltridecanol.

4. Triton K-60 is lauryl dimethylbenzyl ammonium chloride; Triton NE, organic polyether alcohol.

5. Aerosol OT is dioctyl ester of sodium sulfosuccinate.

6. Roccal alkyl dimethylbenzyl ammonium chloride.

serum mixed with pectin. Mixtures of serum and polyvinyl alcohol with Aerosol OT, on the other hand, were free from flocculation, while mixtures of serum and polyvinyl alcohol containing, respectively, Tergitol Penetrants 4 and 7, Roccal and Triton K-60 showed flocculation only in a concentration of 10 parts serum and 1 part polyvinyl alcohol. The addition of these detergents evidently had aided in the stabilization of the colloidal polyvinyl alcohol–serum mixtures, reducing their vibratory lability.

A similar stabilizing effect of detergents (Tergitols, lecithin, dehydrocholic acid) was seen in experiments with mixtures of serum and a 5 per cent solution of gelatin observed under identical experimental conditions.

5. *Serum of Cholesterol-Fed Rabbits and Dogs With and Without Detergents, Cyanides, Iodides and Thioureas.*—An investigation of the effect of vibration and of additions of colloid-active agents on the serum of animals receiving considerable amounts of cholesterol with their food represented the next logical step

TABLE 2.—*Total Serum Cholesterol and Cholesterol Ester Values of Rabbits Fed Considerable Amounts of Cholesterol*

Rabbit	Appearance	Total Cholesterol, Mg. per 100 Cc.	Cholesterol Esters, Mg. per 100 Cc.
98	Milky	469	245
99	Milky	1,061	530
100	Milky	428	235
101	Milky	1,020	571
102	Milky	1,051	632

TABLE 3.—*Flocculation Observed in Shaken Serum of Rabbits Fed Cholesterol in Oil*

Rabbit	Undiluted Serum	Diluted 1:10
98	Trace	+
99	+	+
100	0	0
101	Trace	Trace
102	+	+

in this study. Detergents, iodides, thiocyanides and thiourea compounds were employed in these tests. The rationale for the use of detergents and iodides has been given. Thiocyanides exert a preventive action on experimental cholesterol atheromatosis, as do iodides, and influence the colloidal dispersion of cholesterol.[7] The testing of thiourea compounds was suggested by the fact that these substances are chemically related to thiocyanides and elicit functional and anatomic hyperactivity of the thyroid gland of a type analogous in many respects to that produced by iodides and thiocyanides.

The hypercholesteremic serums of 5 rabbits which had received daily for several months 0.5 Gm. of cholesterol in 15 cc. of corn oil were used for this purpose. The values of total serum cholesterol and of cholesterol esters at the time of the testing are given in table 2.

Undiluted serum of these rabbits and serum diluted with isotonic solution of sodium chloride at a ratio of 1 : 10 were shaken for thirty minutes. Unshaken controls remained unchanged. The shaken specimens showed the reactions listed in table 3.

7. Soerensen, S. P. L.: Kolloid Ztschr. **53**:306, 1930.

In a second set of tubes, to 2 cc. of each of these serums was added 0.1 cc. of a 0.1 per cent watery solution of, respectively, potassium thiocyanide, potassium iodide, lithium iodide, cetylpyridinium iodide, Triton NE, Triton K-60, Aerosol OT and Roccal. The tubes were shaken for thirty minutes. Unshaken tubes with the same contents served as controls. These were always free from flocculation. The results in the shaken tubes are given in table 4.

TABLE 4.—*Flocculation Observed in Shaken Serum of Cholesterolized Rabbits with Additions of Colloid-Active Agents*

Rabbit	Potassium Thiocyanide	Potassium Iodide	Lithium Iodide	Cetylpyridinium Iodide	Triton NE	Triton K-60	Aerosol OT	Roccal
98	+	4+	+	4+	Trace	3+	+	3+
99	3+	3+	2+	+	4+	4+	+	+
100	+	0	3+	Trace	+	3+	Trace	0
101	2+	+	4+	3+	2+	2+	0	Trace
102	2+	4+	2+	2+	Trace	Trace	2+	2+

It is obvious that the addition of the colloid-active agents accentuated in general the vibratory lability of the serum of the cholesterol-fed rabbits. The effect was least pronounced with Aerosol OT.

The fact that the degree of accentuation did not run parallel to the total serum cholesterol level suggested a study of the influence of the concentration of the surface-active agents on the vibratory lability of the lipemic serum. The serum of rabbit 99 was used in subsequent experiments in which 2 cc. of serum was mixed with 0.1 cc. of one or another of the surface-active agents in concentrations of 1:1,000, 1:5,000, 1:10,000, 1:20,000, 1:50,000 and 1:100,000. The following agents were tested: potassium thiocyanide, guanidine thiocyanide, ethylene thiocyanide, lithium iodide, allylthiourea, 5-diethylthiourea, *n*-methyl thiourea and 5-aminouracil. The results are presented in table 5.

TABLE 5.—*Vibratory Lability of Lipemic Rabbit Serum with Additions of Graded Amounts of Colloid-Active Substances*

Substance	Flocculation Observed in Mixture with Given Ratio of Colloid-Active Substance to Serum					
	1:1,000	1:5,000	1:10,000	1:20,000	1:50,000	1:100,000
Potassium thiocyanide	+	0	4+	2+	0	+
Ammonium thiocyanide	+	0	4+	Trace	0	4+
Guanidine thiocyanide	+	Trace	4+	3+	0	2+
Ethylene thiocyanide	+	0	4+	+	0	4+
Lithium iodide	2+	Trace	4+	2+	0	3+
Allylthiourea	0	0	0	2+	Trace	0
Diethylthiourea	2+	+	2+	Trace	3+	Trace
n-Methylthiourea	+	0	0	0	0	4+
5-Aminouracil	Trace	0	0	Trace	2+	3+

The recorded results (table 5) indicate that the various agents used exert a definite effect on the vibratory lability of serum of cholesterolized rabbits. In some concentrations they may stabilize it and in others they may accentuate it. In the case of the thiocyanides these opposite effects seem to alternate with decreasing concentrations in wavelike fashion.

In many of these experiments an additional set of tubes with the same contents as the aforementioned ones was shaken. However, in this set either the

tubes were filled completely, leaving no air space between the cork and the liquid, or the liquid was overlaid with heavy liquid petrolatum, which excluded any contact of the serum mixture with the air and thus any formation of foam. In none of these specially treated tubes was there evidence of flocculation, an observation indicating that contact with air is essential for the production of flocculation.

In one of the experiments with potassium thiocyanide, a chemical analysis was made for the total nitrogen and the total cholesterol content of the supernatant serum and of the floccules obtained as sediment after centrifugation. It was found that the sediment contained about five times as much nitrogen as an equivalent amount (volume) of supernatant serum (5.8 against 28.9 Gm. of protein per hundred cubic centimeters) and that the sediment contained more than twice as much cholesterol as the supernatant serum (320 mg. against 750 mg. per hundred cubic centimeters). This observation suggested that the floccules were composed of a mixture or condensation product of proteins and cholesterol.

In a final experiment tests were made of the vibratory lability of the serum of 4 normal stock dogs, of 2 dogs which had received daily for four months 5 Gm. of cholesterol plus 5 Gm. of dried total ox bile in 120 cc. of corn oil and of 2 dogs which had been fed daily for three months 5 Gm. of cholesterol plus 15 Gm. of lecithin in 120 cc. of corn oil. Undiluted serum was used and serum diluted with saline solution in ratios of 1:1, 1:2, 1:4 and 1:10, as well as diluted with equal parts of a watery solution of potassium thiocyanide in concentrations of 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 0.005, 0.00025, 0.001, 0.0005, 0.00025 and 0.0001 per cent. Total serum cholesterol and ester cholesterol were determined at the same time in the 4 cholesterol-fed dogs: In the cholesterol-bile dogs, the total serum cholesterol was 246 mg. (dog 1938) and 342 mg. (dog 1947) per hundred cubic centimeters, while the ester cholesterol was 57 mg. (dog 1938) and 61 mg. (dog 1947) per hundred cubic centimeters. In the cholesterol-lecithin dogs, the total serum cholesterol was 124 mg. (dog 1878) and 118 mg. (dog 1994) per hundred cubic centimeters, while the ester cholesterol was 50 mg. (dog 1978) and 54 mg. (dog 1994) per hundred cubic centimeters.

After shaking 2 cc. of the serum or of the various mixtures, there were no changes in the tubes containing the serum of the 4 normal dogs. Mild flocculation was found in the cholesterol-lecithin serum containing additions of potassium thiocyanide solution in concentrations upward of 0.025 per cent. The cholesterol-bile serum, on the other hand, exhibited flocculation not only in the higher saline dilutions (1:3 and upward in dog 1947; 1:10 in dog 1938) but also in the tubes containing additions of solutions of potassium thiocyanide. The flocculation was of mild to moderate degree in the serum of dog 1938 and was found with potassium thiocyanide concentrations of 0.5 to 0.005 per cent. In the tubes containing the serum of dog 1947, which had the highest serum cholesterol content, the precipitation started at a potassium thiocyanide concentration of 0.25 per cent, increased in severity in the subsequent lower concentrations, reaching a peak between concentrations of 0.025 and 0.0025 per cent and decreasing progressively in intensity toward the lowest concentration, where it was of minimal extent.

The observations reported indicate that both the cholesterol content of the serum and the concentration of potassium thiocyanide exert a definite influence on the vibratory lability of the serum colloids, including the colloidally dispersed cholesterol.

Chemical Studies of the Blood of Dogs Fed Cholesterol.—In attempts to obtain additional information on the physicochemcial changes which apparently

take place in the plasma of animals fed a high cholesterol-fat diet, chemical studies of the serum of dogs were made to determine the immediate effects on the various lipid fractions of the serum of meals containing cholesterol in oil either alone or in combination with protein (100 Gm. of beef or fish), lecithin (5 Gm. and 15 Gm.) or dried bile (5 Gm.). Blood was withdrawn from the jugular vein three, four, five, seven, twenty-four, twenty-eight and forty-eight hours after such a meal for this purpose. Eighteen dogs were used in the investigation, and 38 tests were made on them.

In 8 of the 18 dogs a prolonged course of cholesterol in oil feeding was subsequently undertaken. Four of these dogs received 5 Gm. of cholesterol plus 5 Gm. of lecithin dissolved in 120 cc. of corn oil mixed with Purina dog chow [8] every other day in addition to their ordinary ration of dog chow. Two were fed this diet for five months and the other 2 for six months. All were then killed by an intravenous injection of a 4 per cent formaldehyde solution. Two dogs received every day for three months 5 Gm. of cholesterol plus 15 Gm. of lecithin in 120 cc. of corn oil. Two dogs were fed daily for three months 7 Gm. of cholesterol plus 7 Gm. of dried ox bile dissolved in 120 cc. of corn oil. The blood of the dogs of the first group was studied every two weeks, while that of the dogs of the second and third groups was studied only during the first month and again directly before they were killed.

The following items were determined: Total serum cholesterol, total ether-extractable cholesterol, ester cholesterol, ether-extractable ester cholesterol, fatty acids, phospholipids, in some instances also total serum calcium, ionizable calcium and nonprotein nitrogen. Hematologic studies were conducted at the same time covering the following items: hemoglobin, erythrocytes, leukocytes, erythrocytic fragility, coagulation time, prothrombin time, sedimentation rate, packed red cell volume, vibratory serum lability and appearance.

A Lumetron photocolorimeter was used for the chemical studies of the blood, with the methods adapted for this apparatus. In the preparation of the ether extracts of total and ester cholesterol 1 cc. of serum was shaken in a 25 cc. volumetric flask with 15 cc. of ether. The content of the flask was then brought to a boil and allowed to boil for a few seconds. After it had cooled and been diluted to make 25 cc., 9.5 cc. of it was used for the determination of total cholesterol and 14.5 cc. for that of ester cholesterol, the digitonin method being employed.

(*a*) Hematic Observations: The serum was milky after a cholesterol-oil meal for a period of up to twenty-eight hours, the peak of turbidity being shown at four to six hours after ingestion. In the majority of dogs there was a reduction of the number of erythrocytes during the first twenty-four hours after the test meal, but in only 4 of them did the number drop by more than 1,000,000 cells. The amount of hemoglobin and the volume of packed red cells underwent fluctuations paralleling those of the erythrocytes. While the fragility tests revealed variations in the osmotic resistance of the erythrocytes, these reactions remained within normal limits and showed no relation to the fluctuations in the number of red cells. The number of leukocytes, the prothrombin time and the sedimentation rate did not exhibit any significant changes. The coagulation time, on

8. According to the Ralston Purina Company, the ingredients of the chow are as follows: meat meal, dried skim milk, riboflavin, carotene, cod liver oil, brewers' dried yeast, wheat germ, corn grits, wheat cereal, corn cereal, dried beet pulp, molasses, steamed bone meal and iodized salt. The chemical analysis shows: protein, 23 per cent, fat 5 per cent, fiber 4 per cent, ash 7 per cent, nitrogen-free extract 54 per cent and moisture 7 per cent.

the other hand, was distinctly shortened during the first seven hours, being reduced to about one fourth of its original value. The peak of this reduction was usually at the five hour test; occasionally it was seen at the three hour test and sometimes as late as the twenty-four and twenty-eight hour tests. In 2 dogs which were fed 3 Gm. of cholesterol in 120 cc. of corn oil there were, however, no appreciable deviations from the original values in theses respects. The tests for vibratory lability revealed no precipitation in any instance during the acute phase of the administration of cholesterol in oil.

Four dogs which were fed daily 5 Gm. of cholesterol, 5 Gm. of lecithin and 120 cc. of corn oil mixed with Purina dog chow, were studied at weekly intervals for a period of two and a half months.

The hemoglobin content fluctuated within normal limits in 2 of the dogs, showed in a third dog a mild increase during the first eight weeks (peak of increase 3.5 mg. per hundred cubic centimeters) and was definitely elevated in the fourth dog during the same period, exhibiting a peak increase of 5 mg. per hundred cubic centimeters above the original figure. The number of erythrocytes varied within normal limits in the first 3 dogs mentioned and revealed a moderate increase (up to 1,800,000 cells) in the fourth dog. There were no significant changes in the number of leukocytes in any of the 4 dogs. The osmotic resistance of the erythrocytes varied within the normal range without displaying any definite or consistent trend. The coagulation time remained within the normal range. The prothrombin time exhibited in all dogs a definite tendency toward elevation, becoming increased from an original level of 8.2 to 11.9 seconds to peak levels of 19.6 to 22 seconds. The sedimentation rate remained unaffected. Tests of the vibratory lability of the serums consistently showed no precipitation.

The hematic studies did not render any significant information with the possible exception of the increase in number of erythrocytes and the elevation in the prothrombin time during the chronic phase and the reduction of the clotting time and the drop in number of erythrocytes during the acute phase.

(*b*) Chemical Observations: The chemical studies of the blood showed that there was in some but not in all dogs a moderate elevation of the total cholesterol from four to twenty-four hours after the ingestion of the cholesterol in oil. This reaction was most marked and most often seen in dogs which received lecithin with the cholesterol in oil. The peak value reached in any of the dogs during the acute reactive phase was 275 mg. of total cholesterol per hundred cubic centimeters of serum. In all other instances the values did not rise above 240 mg. The total ether-extractable fraction, on the other hand, increased in almost all cases appreciably and often considerably above the original values (increases of from two to ten times). These reactions lasted in general during the first twenty-four hours, reaching their peak at the five hour test, but appeared sometimes at the three hour test and were occasionally still present at the forty-eight hour test. While the average value of total ether-extractable cholesterol was 7.78 mg. per hundred cubic centimeters, ranging from 0 to 30 mg. per hundred cubic centimeters in the individual cases, the figures after ingestion were in general above 10 mg. and rose as high as 82 mg. per hundred cubic centimeters.

The serum ester cholesterol values followed in general the pattern set by the total cholesterol values; that is, there was in some cases an increase of ester cholesterol content above the original value. The ether-extractable fraction of ester cholesterol, on the other hand, increased usually appreciably during the first twenty-four hours, reaching a peak at the four to seven hour tests. While

the average ether-extractable fraction of ester cholesterol amounted to 2.1 mg. per hundred cubic centimeters, having a range of 0 to 11 mg., the values were elevated as high as 39 mg. and were quite often between 8 and 20 mg. per hundred cubic centimeters after ingestion of cholesterol in oil.

The serum phospholipid values moved in irregular fashion without following a definite pattern.

The total fatty acids were in general definitely increased by 50 to 200 per cent at the three to seven hour tests.

The determinations of total ionizable serum calcium and of nonprotein nitrogen showed an increase of the nonprotein nitrogen values during the digestive phase when the cholesterol in oil was given together with meat or fish. There were no appreciable or significant fluctuations in the calcium values.

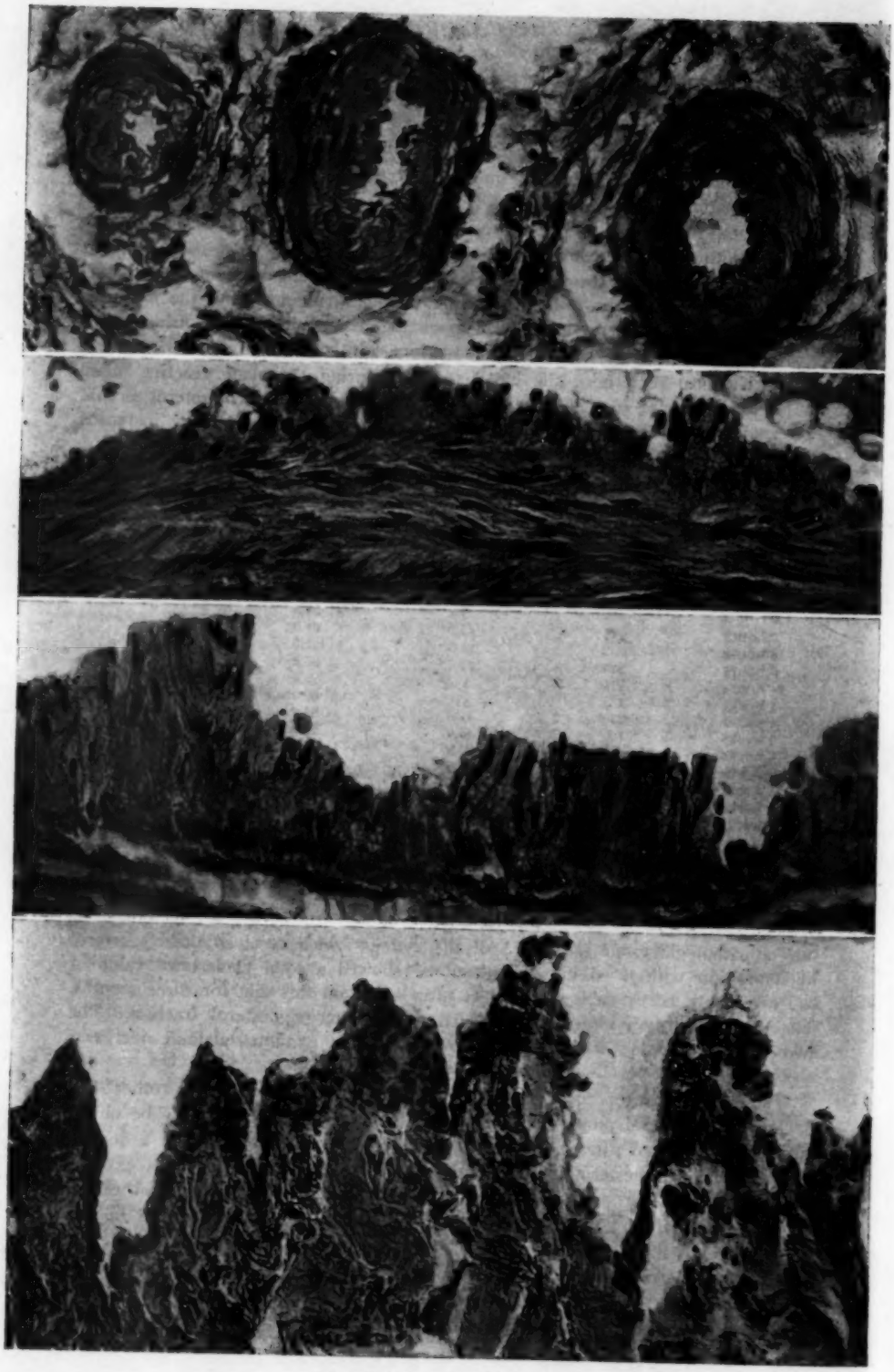
The chemical pattern of the blood revealed during the acute reactive phase was followed during the chronic phase of administration of cholesterol in oil. The total serum values moved in general within normal limits. They increased

TABLE 6.—*Total Cholesterol and Cholesterol Ester Values of the Serum of Dog 1827, Which Was Fed Cholesterol and Lecithin in Its Food*

Time	Total Cholesterol, Mg. per 100 Cc.	Ether-Extractable Total Cholesterol, Mg. per 100 Cc.	Cholesterol Ester, Mg. per 100 Cc.	Ether-Extractable Cholesterol Ester, Mg. per 100 Cc.
0	138	5.1	53.5	0
3 hours	245	45.9	61.0	12.7
4 hours	235	25.5	61.0	5.1
5 hours	240	33.2	66.3	7.6
24 hours	275	7.4	73.9	0
2 weeks	132	5.1	56.0	2.5
4 weeks	240	41.0	82.0	5.1
6 weeks	120	45.0	24.0	3.0
8 weeks	115	20.0	42.0	6.8
10 weeks	112	28.0	49.0	5.1
12 weeks	189	8.0	100.0	0
14 weeks	194	12.0	0	0
16 weeks	184	15.3	63.0	5.1
18 weeks	173	10.2	89.0	0.2
21 weeks	204	6.3	89.0	2.5
23 weeks	190	7.0	122.0	3.0
25 weeks	163	6.0	61.0	2.0

into a pathologic range only in 1 of the 2 dogs which received the cholesterol in connection with dried bile. The animal showed a total cholesterol value of 342 mg. per hundred cubic centimeters after being on this diet for three months. The same stability was found in regard to the ester cholesterol fraction. The two ether-extractable fractions, on the other hand, exhibited definite increases above the normal level on numerous occasions.

Table 6 illustrates these reactions. Dog 1827 was fed every other day 5 Gm. of cholesterol plus 5 Gm. of lecithin in 120 cc. of corn oil. The observations indicate that after a cholesterol-lecithin oil meal there is often a definite increase of the cholesterol fraction not bound to proteins and, therefore, extractable with ether. A small part of this fraction consists of cholesterol esters. In 101 of a total of 129 examinations in which the examined serum was lipemic the ether-extractable fraction of total serum cholesterol exceeded 10 mg. per hundred cubic centimeters. Among 72 determinations on clear serum, on the other hand, only 15 gave a value of ether-extractable total serum cholesterol above 10 mg. per hundred cubic centimeters. This observation suggests that



(See legend on opposite page)

the degree of colloidal instability of the serum cholesterol as represented by the ether-extractable fraction has some connection with the lipemic character of the serum after the ingestion of cholesterol in oil. However, it is apparent that other factors must play a role in the production of this phenomenon.

Anatomic Observations.—The 8 dogs which were subjected to prolonged feeding with the various cholesterol-oil mixtures and which were killed at the end of the experimental period by an intravenous injection of a 4 per cent formaldehyde solution were examined post mortem. There were no significant macroscopic changes of the internal organs with the exception of general obesity, especially characterized by large amounts of subcutaneous and retroperitoneal fat tissue. The liver of 1 dog had a brown-yellow color. Six of the dogs were 1½ to 2½ years old; the seventh was 6 months old and the eighth 4½ years old.

A histologic examination was made of each of the following organs: brain, hypophysis, thyroid and parathyroid glands, lung, heart, liver, pancreas, spleen, adrenal gland, kidney, testis, uterus, vena cava inferior, thymus, duodenum, bone marrow (sternum), aorta, brachial arteries, carotid arteries, iliac arteries and various small branches of the abdominal aorta. For microscopic study the vessels were cut into numerous narrow rings.

With the exception of the acute circulatory reactions elicited by the injection of the formaldehyde solution, the internal organs were essentially free from abnormal changes attributable to the dietary management with cholesterol. One of the dogs which were fed cholesterol plus dried bile exhibited a severe fatty infiltration of the liver cells. The medium-sized intrarenal vessels of this animal showed local medial hyalinizations (fig. 1). The aortas and arteries of the 4 dogs fed cholesterol and lecithin in oil were entirely free from any atheromatous or foam cell lesions with the possible exception of a dog which revealed small foci of proliferated and swollen endothelial cells in the pulmonary artery (fig. 2). There were only calcified hyaline areas in the media of the aorta at the distal end of the aortic bulb. In addition to a similar lesion of the aorta, in 1 of the 2 dogs which received cholesterol in oil there was a small focus in the ascending part of the aorta in which the endothelial cells were swollen and cuboidal or columnar and lined the aortic wall in a stratified or palisade-like arrangement (fig. 3). The vena cava inferior of this animal showed hyaline intimal thickening and calcification of the elastic membrane. There were no abnormal alterations in the vessels of the second dog belonging to this series.

The aorta of 1 of the 2 dogs fed cholesterol plus bile exhibited in its ascending part subintimal hyalinizations of the media with small scattered deposits of blue-stained calcium granules embedded in the hyaline matter. The vena cava inferior of this dog had in places an endothelial lining which was stratified or formed tonguelike processes consisting of swollen cuboidal cells (fig. 4). The ascending part of the aorta of the second dog also revealed local medial hyalini-

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EXPLANATION OF PLATE.

Fig. 1.—Small renal arteries with thickened and locally hyalinized walls.

Fig. 2.—Pulmonary artery with proliferated and swollen endothelial cells.

Fig. 3.—Cuboidal and columnar endothelial cells forming small cushions in the aorta.

Fig. 4.—Vena cava inferior with tonguelike proliferations of swollen endothelial cells.

zations with bluish granular deposits. Here, as well as in other rings, there were areas composed of stratified arrangements of swollen cuboidal or columnar endothelial cells forming pointed processes.

COMMENT

The analysis of the various observations made in these experimental studies reveals the fact that the conditions controlling the vibratory lability of the plasma are highly complex. It was shown that numerous factors may cause the appearance of floccules in serum exposed to shaking, while others counteract such precipitations. The basic fact, however, is apparent, that the development of such precipitates can be elicited only when the surface of the serum mixtures is exposed to air. Thus the production of foam and thereby the formation of film are permitted. When this mechanism is excluded by complete filling and stoppering of the tubes or by overlaying of the liquid content with heavy liquid petrolatum flocculation is consistently prevented. It was observed that the precipitates developed not only in serums to which macromolecular substances or colloidally active agents were added but also in serums of animals showing dietary hypercholesteremia and occasionally also in serums of "normal" animals.

The ease with which floccules appear in the serum of animals given injections of solutions of polyvinyl alcohol or gelatin has been previously noted incidental to in vivo experiments [9] with other agents. The present in vitro investigations confirm those observations. It is remarkable, on the other hand, that other atheromatogenic macromolecular substances, such as methyl cellulose and pectin, did not respond with the formation of floccules when shaken with normal rabbit serum. It was observed, however, that the serum of hypercholesteremic rabbits and of dogs fed large amounts of cholesterol showed floccular precipitates on shaking. Thus these observations support only in part the theory of the significance of vibratory influences in the production of atheromatous lesions. The absence of air in the blood circulating in the vascular tree eliminates the important factor of film formation in the interface for the production of precipitates in vivo. These consist of cholesterol-protein complexes.

The various detergents tested apparently increased the vibratory lability of normal serum. The iodides, on the other hand, were in general without any definite effect in this respect. When added to mixtures of serum and solutions of macromolecular colloids some of the detergents (Tergitol Penetrants 4 and 7, Roccal and Triton K-60) seemed to exert a stabilizing influence on the mixtures previously found to be relatively unstable (serum plus polyvinyl alcohol, serum plus

9. Footnotes 1*a* and 2*c*.

gelatin). The type of detergent, as well as the colloidal medium, thus plays an important role in determining the effect of vibratory influences on colloidal stability. Studies on hypercholesteremic serum showed that the concentration of the colloid-active agents (thiocyanides, thioureas) is also a deciding factor in this respect, as the colloidal vibratory lability of such serum was either intensified or diminished, depending on the concentration of the various thiocyanides and thioureas added. The present investigations, however, are not sufficiently elaborate for deciding whether or not variations in the status of the plasma colloids are reflected by definite patterns of colloidal vibratory lability having diagnostic significance.

The positive findings in the serum of hyperlipemic rabbits and in the serum of dogs with quantitatively normal total cholesterol and ester cholesterol which ingested large amounts of cholesterol in oil indicate that not only an excessive amount of cholesterol in the serum but also the physicochemical status of the serum cholesterol as influenced by dietary intake plays a decisive role in the vibratory lability of this colloidal liquid. The frequent and considerable increase of the ether-extractable fraction of free and of bound cholesterol observed in dogs which had ingested cholesetrol in oil suggests that this factor may be important in this respect, as the ether-extractable portion of cholesterol is that fraction which is not bound in some way to the plasma proteins and which is, thus, colloidally not stabilized (Soerensen[7]; Handovsky[10]). A relative increase of the ether-extractable fraction of serum cholesterol, therefore, may indicate an elevation of a colloidally labile fraction of cholesterol, which may occur in the absence of any significant and abnormal rise in the levels of total and ester serum cholesterol. It seems to be possible that such shifts in the physicochemical status of cholesterol in the serum under the influence of dietary factors are responsible for the appearance of floccules in the serum that is exposed to shaking and may be of significance for the production of dietary atheromatosis.

If this phenomenon may be taken as an index of increased vibratory colloidal lability of the serum, it may provide a plausible explanation for the appearance of the localized proliferations of swollen endothelial cells often forming cellular processes and coatings in the aorta, the pulmonary artery and the vena cava inferior of several of the dogs kept for several months on a high cholesterol diet. These lesions appeâr to be early atheromatous manifestations, as they resemble those seen in rabbits after cholesterol feeding as well as those observed in dogs given injections of various macromolecular substances, particularly hydroxyethylcellulose.[1b] They were most marked in the dogs which received

10. Handovsky, H.: Klin. Wchnschr. **3**:1354, 1924.

for only three months 7 Gm. of cholesterol plus 7 Gm. of dried ox bile in 120 cc. of corn oil, while they were absent in all but 1 of the dogs which received a cholesterol-lecithin-oil mixture.

These observations are in general agreement with those made recently by Member, Bruger and Oppenheim,[11] who found that the combined feeding of cholesterol and bile acids to rabbits favored the development of a high cholesterol content of the blood and of the aorta and aortic atheromatosis. Steiner and Domanski,[12a] on the other hand, were unsuccessful in eliciting in dogs kept for forty-four to fifty-six weeks on a diet containing 100 Gm. of egg yolk powder daily any atheromatous arterial lesions. The egg yolk powder consisted of lecithin 14.4 per cent, cholesterol 8 per cent and fat 40.6 per cent. Experiments of the same investigators have shown that the administration of soybean lecithin to patients caused hypocholesteremia, while Kesten and Sibowitz [13] reported that in rabbits receiving soybean lecithin together with cholesterol only minimal or no atheromatosis developed. This anti-atheromatogenic effect of lecithin may be due to the fact that lecithin tends to keep cholesterol in the dispersed phase and thus counteracts its flocculation and deposition.

Mention may be made in this connection of the observations of Frey [14] concerning shifts in the globulin-albumin ratio in the tissues of the vascular walls preceding the deposition of cholesterol and calcium and their influence on this phenomenon through the resulting disturbance of the colloid equilibrium. Similar factors are apparently responsible for the reduced stability of cholesterol in the serum of persons with atherosclerosis. Frey found that when 0.1 Gm. of cholesterol is added to 5 cc. of serum obtained from normal persons, there is usually a distinct increase in the cholesterol content of the serum. When, on the other hand, the serum of atherosclerotic persons is used, there occurs a definite decrease in the cholesterol content of the serum as cholesterol is precipitated from the serum. Such reductions may amount to more than 100 mg. of cholesterol per hundred cubic centimeters of serum and indicate that the serum of atherosclerotic persons contains a labile fraction of cholesterol.

Mention may be made finally of some of the hematic reactions observed in the dogs kept on a cholesterol diet. The transitory decreases

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11. Member, S.; Bruger, M., and Oppenheim, E.: Arch. Path. **38**:210, 1944.

12. Steiner, A., and Domanski, B.: (*a*) Am. J. M. Sc. **201**:820, 1941; (*b*) Proc. Soc. Exper. Biol. & Med. **55**:236, 1944.

13. Kesten, H. D., and Sibowitz, R.: Proc. Soc. Exper. Biol. & Med. **49**: 71, 1942.

14. Frey, W.: Verhandl. d. deutsch. Gesellsch. f. inn. Med. **51**:51, 1939.

in the number of erythrocytes found in the majority of the dogs after a single meal with added cholesterol in oil are possibly attributable to the massive influx of fatty acids into the blood, causing there increased osmotic fragility (Johnson, Freeman and Longini [15]; Davis [16]; Johnson, Freeman, Longini and Loewy [17]; Longini and Johnson [18]), an effect which was not unequivocally demonstrable in the present experiments. It is possible that such a destructive action on the erythrocytes may be the cause of the simultaneous considerable shortening of the coagulation time observed. The significant increase in the number of erythrocytes found in 2 of the 4 dogs subjected to prolonged administration of cholesterol and lecithin in oil may represent a phenomenon compensatory for the increased erythrocytic destruction causing overstimulation of the bone marrow, or may be the result of anoxia of the marrow resulting from the circulatory effect of the choline fraction contained in lecithin, as proposed by Davis.[16] The elevation of the prothrombin time observed in all dogs of this series, on the other hand, may be related to functional reactions of the liver brought about by the abnormal intake and the hepatic metabolization of the various lipids.

SUMMARY

Floccules appear in normal rabbit serum which has been mixed with a solution of polyvinyl alcohol and a solution of gelatin, on shaking. Identical reactions occur when the hyperlipemic serum of rabbits fed cholesterol and the normally lipemic serum of dogs fed cholesterol are shaken with solutions of various colloid-active substances (thiocyanides, thioureas) or are diluted with isotonic solution of sodium chloride. Detergents added to normal rabbit serum tend to increase the vibratory lability and often elicit formation of floccules composed of protein-cholesterol complexes. Certain detergents, on the other hand, when added to mixtures of serum and polyvinyl alcohol or gelatin exert a stabilizing effect on the colloidal equilibrium of the mixtures. The vibratory lability of hypercholesteremic rabbit serum is either intensified or diminished by the addition of thiocyanides and thioureas, depending on the relative concentrations of these agents. The development of a precipitate is bound to the formation of a film in the foam produced by shaking in the interface of air and liquid. Dogs fed considerable amounts of cholesterol in oil show a definite increase of the ether-

15. Johnson, V.; Freeman, L. W., and Longini, J.: J. A. M. A. **124**:1250, 1944.

16. Davis, J. E.: Am. J. Physiol. **142**:65, 1944.

17. Johnson, V.; Freeman, L. W.; Longini, J., and Loewy, A.: Federation Proc. **3**:22, 1944.

18. Longini, J., and Johnson, V.: Am. J. Physiol. **140**:349, 1943.

extractable fractions of total cholesterol and ester cholesterol, which are probably colloidally less stable than the cholesterol fractions bound to proteins.

Several of the dogs, especially those receiving cholesterol in oil plus dried ox bile dissolved in corn oil, showed localized proliferations and swelling of the endothelial lining of the aorta, the pulmonary artery and the vena cava inferior. These lesions apparently represent early atheromatous changes.

STRUCTURAL CHANGES IN THE THYROID GLANDS OF PATIENTS TREATED WITH THIOURACIL

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THE morphologic changes in the thyroid glands of patients with hyperthryoidism are well enough known so that a fair correlation can be made between structure and function. This situation seemingly ended abruptly when thiouracil was introduced for the treatment of hyperthryoidism.[1] Clinical observations on patients with hyperthyroidism treated with thiouracil are accumulating. Morphologic studies of the thyroid glands of such patients, however, are comparatively few.[2] Yet such studies may shed considerable light on the probable mechanism of, and the principles involved in, the action of thiouracil. In this paper we present the results of observations on 7 patients with marked signs and symptoms of exophthalmic goiter who were so treated as to provide material for a study of the effects on the thyroid gland of thiouracil alone, of strong solution of iodine U.S.P. followed by thiouracil, and of thiouracil followed by strong solution of iodine U.S.P.[2a]

PATIENTS TREATED WITH THIOURACIL ONLY

CASE 1.—E. K., a 48 year old woman, was admitted to the University Hospitals on Sept. 4, 1944. Nine months previously she had noted rapid loss of weight and increased appetite, with subsequent development of nervousness, tremor, excessive perspiration, tachycardia and fatigability. Within six months she had lost 40 pounds (18 Kg.) and was bedfast most of the time due to extreme weakness. On admission, she was extremely nervous, weighed 98 pounds

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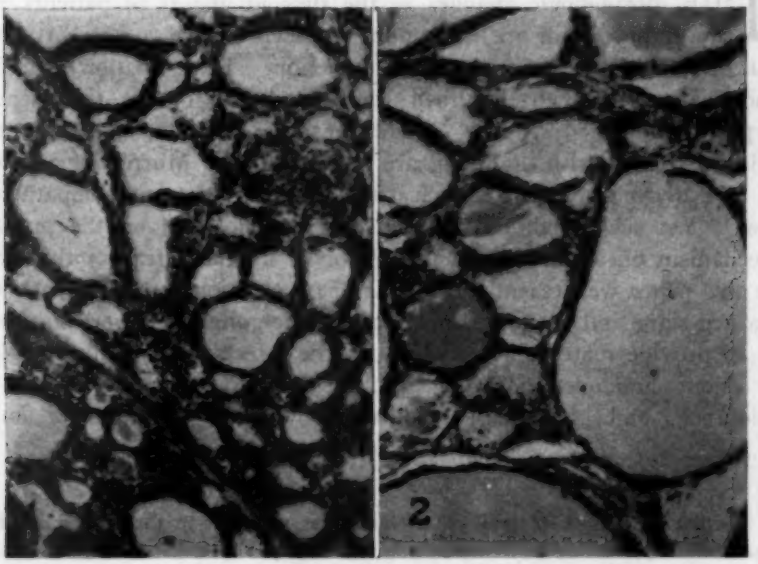
1. Astwood, E. B.: J. A. M. A. **122**:78, 1943.

2. Williams, R. H., and Clute, H. M.: New England J. Med. **230**:657, 1944. Rawson, R. W.; Evans, R. D.; Means, J. H.; Peacock, W. C.; Lerman, J., and Cortell, R. E.: J. Clin. Endocrinol. **4**:1, 1944. Moore, F. D.; Sweeney, D. N., Jr.; Cope, O.; Rawson, R. W., and Means, J. H.; Ann. Surg. **120**:152, 1944. Lozinski, E., and Siminovitch, J.; Canad. M. A. J. **51**:422, 1944. Palmer, M. V.: Ann. Int. Med. **22**:335, 1945.

2a. The thiouracil used in these studies was supplied by the Medical Research Department of the Winthrop Chemical Company, Inc.

(44.5 Kg.), presented a moderately diffuse enlargement of the thyroid gland and a fine tremor and had slight exophthalmos. The pulse rate was 108; the blood pressure was 145 systolic and 76 diastolic, and the basal metabolic rate was plus 58 per cent. Thiouracil was administered orally, 0.6 Gm. daily in divided doses, for three weeks. At the end of this time the basal metabolic rate was plus 25 per cent, the pulse rate 84, and the blood pressure 126 systolic and 72 diastolic. Thiouracil was not available for the next two weeks, during which time the basal metabolic rate rose to plus 58 per cent. Four weeks after thiouracil therapy was resumed (0.4 Gm. daily), the basal metabolic rate was plus 12 per cent, the pulse rate 88, and the blood pressure 130 systolic and 68 diastolic. She had gained only 1 pound. Eight weeks after the beginning of thiouracil therapy (on November 1) subtotal thyroidectomy was performed by Dr. John W. Cavanaugh.



Figs. 1 and 2 (cases 1 and 2).—Microscopic appearance of the thyroid glands of patients treated with thiouracil only. Various-sized acini are seen lined by cuboidal or low columnar cells, with the lumens containing faintly stained colloid, vacuolated and scalloped. × 100.

Description of Specimen.—The thyroid tissue removed comprised both lobes, measuring 6 by 4 by 2.5 and 5.5 by 3.5 by 2 cm. The external surfaces were coarsely lobulated. The posterior surfaces were ragged and had a colloid sheen. On microscopic examination (fig. 1) acini of variable size lined by cuboidal or low columnar cells were seen fairly close to one another, separated by delicate or incomplete septums. The lumens contained faintly stained colloid, vacuolated and scalloped, or were empty. Groups of interacinous cells were numerous in some of the lobules.

CASE 2.—Z. L., a 44 year old Negro housewife, was first admitted to the University Hospitals March 20, 1944. She complained of nervousness and loss of weight with increased appetite. She had had a goiter for five years. Seven months prior to admission she noticed an increase in the size of her neck and

progressive bulging of her eyes. She denied having taken iodine at any time. On admission, the thyroid gland was diffusely enlarged, and she had well developed signs and symptoms of exophthalmic goiter. Her weight was 122 pounds (55 Kg.). The blood pressure was 160 systolic and 65 diastolic, and the heart rate was 144. A mitral and aortic systolic murmur could be heard, which was transmitted to the vessels of the neck and the axilla. The basal metabolic rate was plus 60 per cent. Thiouracil was administered, 0.6 Gm. daily in divided doses, beginning March 25, when her weight was 118 pounds (53.5 Kg.). Forty-nine days later her weight had increased from 118 to 128 pounds (53.5 to 58 Kg.), and the basal metabolic rate was plus 13 per cent, the blood pressure was 160 systolic and 90 diastolic, and the pulse rate was 84. On May 16 bilateral subtotal thyroidectomy was performed by Dr. John H. Robinson.

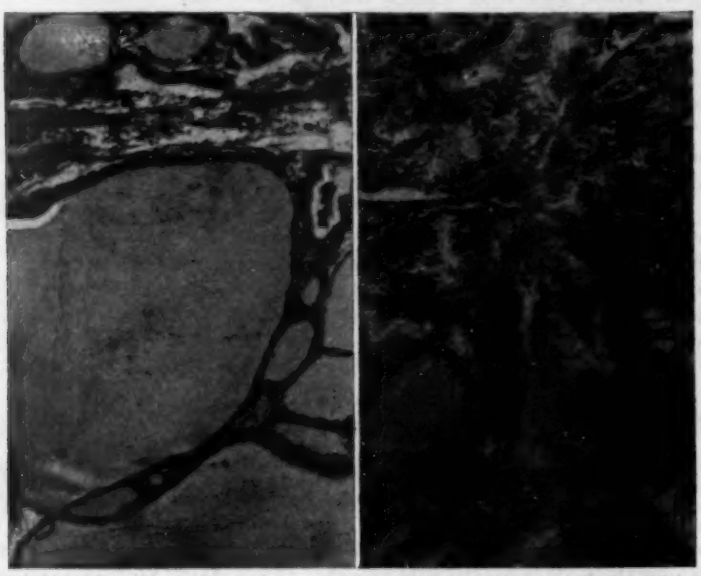
Description of Specimen.—The thyroid tissue removed consisted of both lobes, measuring 4.5 by 3.5 by 3 and 4.5 by 3.5 by 2.5 cm. On each lobe there was a ragged surface, measuring 4 by 2 cm.; the remaining surfaces were nodular and covered with a thin, transparent membrane. The cut surfaces were gray-pink and lobulated, with delicate septums separating the lobules. At one pole there was a circumscribed nodule, 1.5 cm. in diameter, which had a complete capsule. The nodule was pale yellow-pink and had a colloid sheen. On microscopic examination (fig. 2) variable-sized acini were seen in groups, some covering the greater part of a microscopic field. The acini were separated by delicate or incomplete septums, which were lined by flat or cuboidal cells. In other fields the acini were small and were lined by cuboidal or columnar cells and separated by sheets of interacinous cells. Some of the acini contained a light-stained, vacuolated and scalloped colloid. Occasional ones were empty. Spur-like protrusions covered by columnar cells were seen in some of the lumens. In the stroma there were aggregations of lymphocytes, plasma cells and large mononuclear cells.

PATIENTS TREATED WITH STRONG SOLUTION OF IODINE U. S. P. FOLLOWED BY THIOURACIL

CASE 3.—M. H., a 33 year old housewife, was admitted to the University Hospitals March 14, 1943. She had had a goiter for fifteen years, and because of symptoms of hyperthyroidism she had been treated intermittently with strong solution of iodine U. S. P. until about one and one-half years before her admission. At that time the basal metabolic rate was plus 43 per cent. After preparation with strong solution of iodine, on April 7, bilateral subtotal thyroidectomy was performed by Dr. Charles M. O'Leary. When discharged the patient was given strong solution of iodine and instructed to return in four weeks. She failed to return, continued to have symptoms of hyperthyroidism and continued taking strong solution of iodine until her readmission on Feb. 11, 1944. At this time there were recurrent enlargement of the thyroid gland, nervousness, palpitation, marked exophthalmos with pain in the eyes, loss of weight and weakness. She weighed 147 pounds (66.5 Kg.), her pulse rate was 120 and the basal metabolic rate was plus 28 per cent. Thiouracil was administered, 0.6 Gm. daily in divided doses. Eighteen days later the basal metabolic rate had dropped to plus 9 per cent, and she was discharged from the hospital with instructions to take 0.2 Gm. of thiouracil daily. She continued taking the drug until her third admission May 29, when she weighed 167 pounds (75.5 Kg.) and the basal metabolic rate was minus 26 per cent. On June 2, bilateral subtotal thyroidectomy was performed by Dr. Charles M. O'Leary.

Description of Specimen.—The specimen obtained at the first operation (April 7, 1943) consisted of two pieces of thyroid tissue measuring 7 by 4 by 2.5 and 6.5 by 4 by 2 cm. The surfaces were brown-pink, smooth and glistening. The cut surfaces were pink-red, with a meaty appearance and a colloid sheen. On microscopic examination (fig. 3) there were marked variations in the size of the acini, which were lined by flat or low cuboidal cells. Some had infoldings covered with columnar epithelium. The colloid stained uniformly pink. In some areas the septums were delicate and in places incomplete. In other areas there were acini with small lumens and groups of interacinous cells. The septums in places were infiltrated with lymphocytes to a degree almost amounting to lymph follicle formation.

The specimen obtained at the second operation (June 2, 1944) consisted of three globular masses of thyroid tissue measuring 5 by 4 by 3 and 5 by 3.5 by 2.5 and 5 by 2.5 by 2 cm., respectively. One surface of each was raw; the other was



Figs. 3 and 4 (case 3).—Microscopic appearance of the thyroid gland of a patient treated with strong solution of iodine U. S. P. followed by thiouracil. The patient was a 33 year old woman, with a basal metabolic rate of minus 26 per cent at the time of her second operation (fig. 4). × 100.

covered by a delicate membrane. The cut surfaces were pale gray, with no colloid sheen. On microscopic examination various-sized acini, some with elongated, others with round lumens, were seen in a scanty connective tissue stroma (fig. 4). The lumens were empty or contained a slight amount of light-stained colloid. The lining cells were tall columnar or cuboidal. The cytoplasm of many of the cells appeared vacuolated. Sheets of interacinous cells were seen with no lumens. Broad bands of hyalinizing fibrous connective tissue subdivided the lobules. Into some of the lumens protruded infoldings lined by tall columnar cells. In places the cell nuclei were heaped up into several rows. In the larger spaces colloid was more abundant and appeared scalloped and vacuolated.

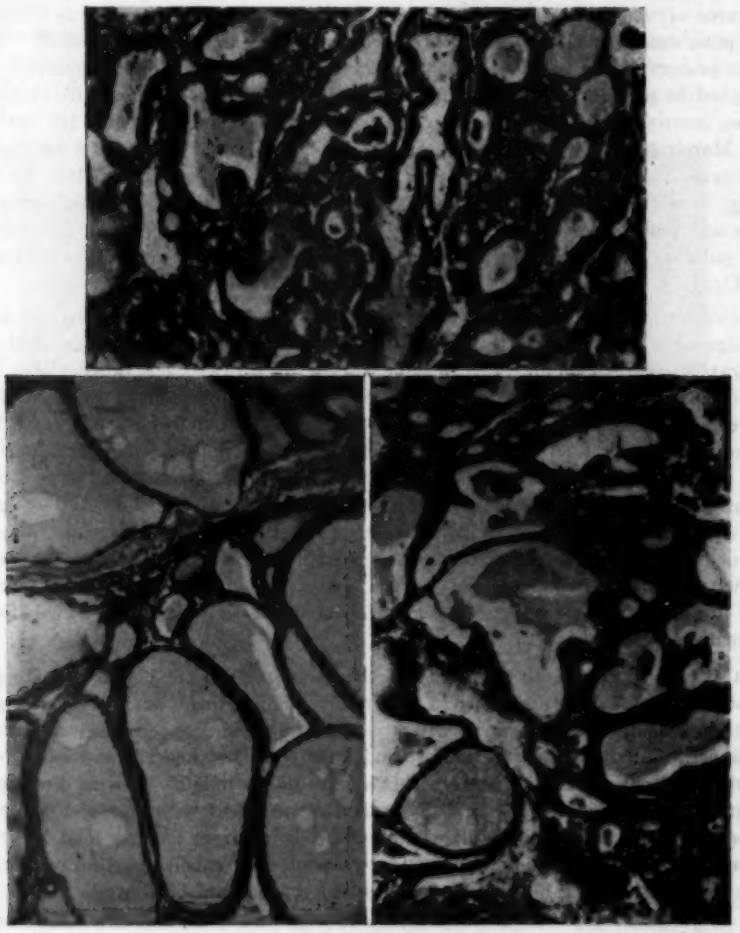
CASE 4.—E. B. S., a 31 year old housewife, was admitted to the University Hospitals March 3, 1944. She had had enlargement of the thyroid gland for twelve years. In 1938 nervousness, loss of weight with increased appetite, tachycardia, intolerance of heat and severe exophthalmos developed. The same year, during a seven month stay in the hospital and after preparation with iodine, the right lobe of the thyroid gland was removed. Improvement was noted until 1940, when her symptoms recurred. Iodine gave some relief for the following year; then objective and subjective symptoms became progressively worse, and for three months prior to admission the symptoms of cardiac decompensation were severe. On admission, the patient's thyroid gland was diffusely enlarged, and she exhibited extreme nervousness, tremor and marked exophthalmos. The heart was enlarged; the pulse rate was 112, the blood pressure was 155 systolic and 55 diastolic. Rales were present in the bases of both lungs, and dependent edema was apparent. She weighed 84 pounds (38 Kg.). Thiouracil was administered, 1 Gm. daily in divided doses, starting March 8, 1944, when the basal metabolic rate was plus 107 per cent. On March 17 the basal metabolic rate was plus 76 per cent, and she had gained 5 pounds (about 2 Kg.). The dose of thiouracil was then reduced to 0.6 Gm. daily. On April 10 her weight was 109 pounds (49.5 Kg.), the basal metabolic rate was plus 29 per cent, the blood pressure was 140 systolic and 80 diastolic and the pulse rate 80. On April 17 bilateral subtotal thyroidectomy was performed by Dr. L. J. Starry.

Description of Specimen.—The specimen consisted of one lobe of thyroid gland, measuring 8 by 5 by 3 cm. Its surface was coarsely lobulated and covered by a delicate membrane. One fourth of the surface was raw. The cut surfaces were divided into various-sized gray-pink fields without a colloid sheen. There were four additional portions of thyroid tissue, together measuring 5 by 4 by 2 cm. They resembled the other externally and on their cut surfaces. On microscopic examination (fig. 5) there was marked variation in the size of the acini. Some were elongated and had spurlike infoldings lined by tall columnar cells. The acini contained a light-stained network of fibrillar material with pink-stained colloid, scalloped in places and vacuolated in others. Groups of interacinous cells were seen here and there. The connective tissue stroma was scanty, and in places there were aggregations of lymphocytes, amounting to lymph follicle formation.

CASE 5.—G. W., a 16 year old girl, was admitted to the University Hospitals Sept. 11, 1944. She had noted a progressive enlargement of her thyroid gland with increasing nervousness for two years. Later she experienced an increase of appetite but began to lose weight and noted that her heart beat rapidly. Her mother had observed prominence of her eyes for four months. No medication had been taken. On admission she had marked nervousness with tremor and definite exophthalmos. The thyroid gland was diffusely enlarged, and a loud bruit was heard over each lobe. The heart was not enlarged; the cardiac rhythm was regular and the rate 140; the blood pressure was 150 systolic and 68 diastolic. The basal metabolic rate was plus 53 per cent. Strong solution of iodine U. S. P., 10 minims (0.61 cc.) three times a day, was given for four weeks. At the end of this period her pulse rate was 88, the blood pressure 128 systolic and 68 diastolic, and the basal metabolic rate plus 6 per cent. She had gained 3 pounds (about 1 Kg.). On October 13, after right subtotal lobectomy had been performed by Dr. Charles M. O'Leary, her pulse rate and blood pressure rose to such alarming heights that removal of the left lobe was not attempted. A stormy postoperative period ensued with evidence of thyroid crisis and bronchopneumonia. On the third post-

operative day administration of thiouracil was begun, 0.6 Gm. daily in divided doses. The administration of strong solution of iodine, 30 minims (1.85 cc.) daily, was continued. At the end of three weeks she had gained 10 pounds (4.5 Kg.), the pulse rate was 74, and the basal metabolic rate was plus 1 per cent. Subtotal lobectomy was performed on the left side, November 8, by Dr. Charles M. O'Leary.

Description of Specimen.—The specimen obtained at the first operation (Oct. 13, 1944) consisted of two pieces of thyroid tissue measuring 6 by 4 by 3 and 2 by 1 by 1.5 cm. The external surfaces were coarsely nodular. The nodules varied from 0.5 to 1 cm. in diameter. The cut surfaces had a meaty appearance with a



Figs. 5 (case 4), 6 and 7 (case 5).—Microscopic appearance of the thyroid glands of patients treated with strong solution of iodine U. S. P. followed by thiouracil. Figure 5 shows the appearance of the thyroid gland of a 31 year old woman (case 4) at the time of her second operation, following the administration of thiouracil. Figures 6 and 7 show the appearance of the thyroid gland of a 16 year old girl (case 5) after the administration of strong solution of iodine U. S. P. followed by thiouracil. × 100.

colloid sheen; some of the vesicles were visible and measured up to 0.2 cm. in diameter. On microscopic examination (fig. 6) the acini varied in size and shape. They were lined by low columnar or cuboidal cells, had delicate or incomplete septums and were filled with light-stained or dark-stained colloid. Others contained large mononuclear cells with lightly stained cytoplasm and eccentrically placed nuclei. Sheets of interacinous cells were seen in places. Many of the acini had spurlike infoldings. In the connective tissue stroma in places there were aggregations of lymphocytes amounting to lymph follicle formation.

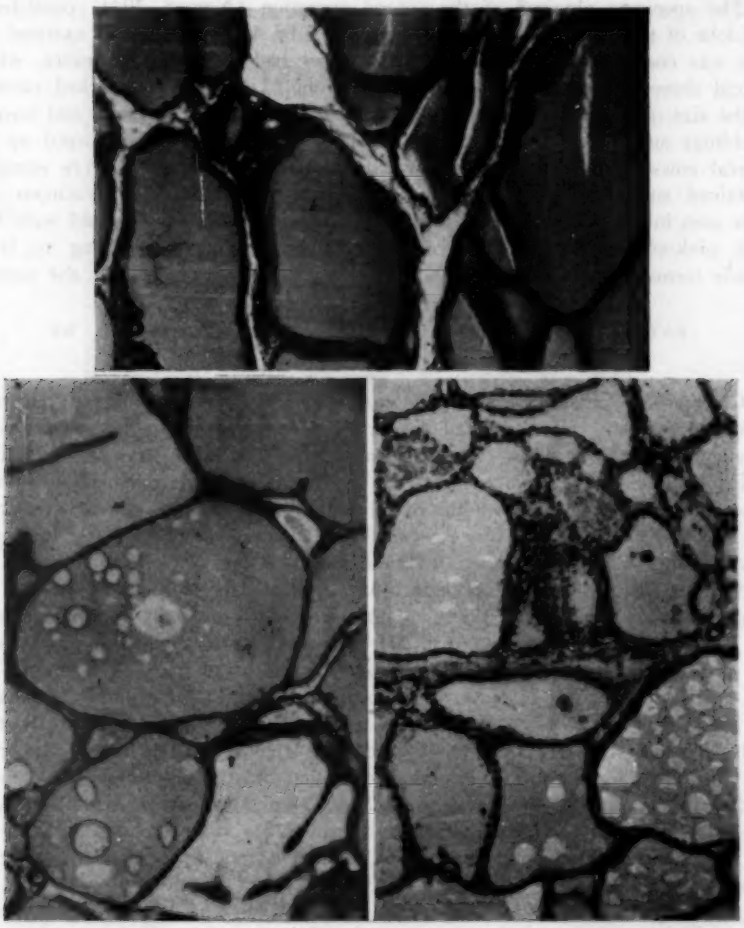
The specimen obtained at the second operation (Nov. 8, 1944) consisted of one lobe of thyroid gland with the isthmus, 6.5 by 4 by 3 cm. The external surface was coarsely lobulated. The cut surfaces had a meaty appearance, with a colloid sheen. On microscopic examination (fig. 7) there was marked variation in the size of the acini in the individual lobules. Some of the acini had frequent infoldings and were lined by tall columnar cells with the nuclei heaped up into several rows. Others were lined by cuboidal or columnar cells, were empty or contained some pink-stained amorphous material. Sheets of interacinous cells were seen in places. Occasional large acini were lined by flat cells and were filled with pink-stained colloid. Aggregations of lymphocytes amounting to lymph follicle formation, with overgrowth of germinal centers, were seen in the septums.

PATIENTS TREATED WITH THIOURACIL FOLLOWED BY STRONG SOLUTION OF IODINE U. S. P.

Case 6.—B. H., a 33 year old housewife was admitted to the University Hospitals March 11, 1944. She had had signs and symptoms of exophthalmic goiter, developing over a period of eighteen months. She had been given strong solution of iodine U. S. P. for two months, this treatment being terminated four months prior to her admission. On admission, her thyroid gland was diffusely enlarged, and she had extreme nervousness, tremor and marked exophthalmos. Her heart was enlarged; the pulse rate was 120, and the blood pressure was 140 systolic and 70 diastolic. She weighed 96 pounds (43.5 Kg.), and the basal metabolic rate was plus 65 per cent. Thiouracil was administered, 0.6 Gm. daily in divided doses, for six weeks, at which time (April 22) the pulse rate was 92, the blood pressure 110 systolic and 65 diastolic, and the basal metabolic rate plus 49 per cent. She had gained 10 pounds (4.5 Kg.). At operation on April 24 (by Dr. L. J. Starry) the pulse rate rose to 140 and the blood pressure to 180 systolic and 100 diastolic. Only the right lobe of the thyroid gland was removed. The use of thiouracil, 0.6 Gm. daily, was continued, together with that of strong solution of iodine, 15 minims (0.92 cc.) three times daily. On May 22 she weighed 117 pounds (53 Kg.), the pulse rate was 88, the blood pressure was 115 systolic and 80 diastolic, and the basal metabolic rate was plus 18 per cent. On June 5 left hemithyroidectomy was performed by Dr. C. E. Clymer.

Description of Specimen.—The specimen obtained at the first operation (April 24) consisted of a lobe of thyroid gland measuring 6 by 3.5 by 2 cm. The surface was coarsely lobulated; half of it was raw and the other half, covered by a thin membrane. The cut surfaces were pale pink and had a colloid sheen. On microscopic examination (fig. 8) there was marked variation in the size of the lobules and of the acini within the lobules. The acini were lined by cuboidal or columnar cells, with occasional spurs forming incomplete septums. The acini contained pink-stained colloid. Groups of interacinous cells were seen in places. Other acini were lined by low cuboidal cells. In the septums there were, here and there, aggregations of lymphocytes amounting to lymph follicle formation.

The specimen obtained at the second operation (June 5) consisted of a lobe of the thyroid gland measuring 5.5 by 4.5 by 2.5 cm. One surface was raw; the other was covered by a delicate membrane and was slightly lobulated. On the cut surfaces there were gray-pink fields having a colloid sheen and separated by delicate pale gray septums. On microscopic examination (fig. 9) various-sized acini lined by low cuboidal cells filled with pale pink-stained colloid were seen close to one another, separated by delicate or incomplete septums. An occasional



Figs. 8 and 9 (case 6) and 10 (case 7).—Microscopic appearance of the thyroid glands of patients treated with thiouracil followed by strong solution of iodine U. S. P. Figure 8 shows the appearance of the thyroid gland of a 35 year old woman (case 6) operated on while her basal metabolic rate was plus 49 per cent after she had received thiouracil for six weeks. Figure 9 shows the appearance of the specimen obtained at the second operation on this woman, following the administration of strong solution of iodine U. S. P. and thiouracil for six weeks. Figure 10 shows the appearance of the thyroid gland of a 37 year old woman (case 7) treated with thiouracil followed by strong solution of iodine U. S. P. × 100.

lumen had infoldings lined by tall columnar cells. The variation in the size of the acini was more marked in some fields than in others. Here and there groups of interacinous cells were seen. In focal areas there were some aggregations of lymphocytes amounting to lymph follicle formation.

CASE 7.—R. N., a 37 year old woman, was admitted to the University Hospitals Oct. 10, 1944. Three months prior to admission she began to suffer from nervousness, restlessness, fatigability and a rapid heart beat. Although her appetite increased, she gradually lost weight and noticed intolerance of heat. One month prior to admission she noticed enlargement of her neck. She had received no medication. On admission, nervousness and a fine tremor were apparent. There was widening of the palpebral fissures with a stare but no exophthalmos. The thyroid gland was moderately and diffusely enlarged. The blood pressure was 130 systolic and 84 diastolic; the pulse rate was 134 and the basal metabolic rate plus 53. Thiouracil was administered, 0.6 Gm. daily, and after one week the basal metabolic rate was plus 35 per cent. She continued to take thiouracil for the next three weeks as an outpatient. On November 7, four weeks after the beginning of the thiouracil treatment, she was readmitted. At this time the basal metabolic rate was plus 20 per cent, the pulse rate 88 and the blood pressure 140 systolic and 80 diastolic. She had gained 2 pounds (about 1 Kg.) and showed marked improvement in subjective symptoms. Strong solution of iodine U. S. P. was administered, 5 minims (0.31 cc.) three times a day, in addition to thiouracil, 0.4 Gm. daily. After six days the basal metabolic rate was plus 6 per cent. On November 17 bilateral subtotal thyroidectomy was performed by Dr. John W. Cavanaugh.

Description of Specimen.—The specimen obtained consisted of both lobes of the thyroid gland with the pyramidal lobe, together weighing 35 Gm. The left lobe was 5 by 3 by 2 cm., the right 6 by 3 by 2 and the pyramidal lobe 2 by 0.6 cm. The external surfaces were coarsely lobulated. The cut surfaces had a colloid sheen and were even. On microscopic examination (fig. 10) there was marked variation in the size of the acini within the individual lobules and also in the size of the lobules. Most of the acini contained a faintly stained colloid or no colloid and were lined by flat cuboidal or columnar cells. The nuclei of the latter in places were heaped up into several rows. Other acini contained pale lavender-stained granular material. In some of the lobules the septums were delicate and incomplete and the covering cells almost flat. Groups of interacinous cells were seen in places. In focal areas there were aggregations of lymphocytes, plasma cells and large mononuclear cells.

COMMENT

All 7 patients when first seen had marked signs and symptoms of hyperthyroidism or exophthalmic goiter. On all, bilateral subtotal thyroidectomy was performed in one or two stages. Two patients had received preoperative therapy consisting of administration of thiouracil only; 3 were treated with strong solution of iodine U. S. P. followed by thiouracil, and 2 were treated with thiouracil followed by strong solution of iodine. In most instances, the intensity of the clinical signs and symptoms was lowered, the pulse and basal metabolic rates were near normal levels and the patients were gaining weight at the time of operation.

The morphologic observations were made on routine preparations stained with hematoxylin and eosin. Sections stained with Haidenhain's azocarmine and by Van Gieson's method were also studied.[3]

In the glands removed following the administration of thiouracil only, the acinous content stained lightly and was decreased or absent, and the cells lining the acini were low or tall columnar. These changes were quite similar to those seen in thyroid glands of untreated patients in an active state of hyperthyroidism.

More illuminating information was obtained from the study of the thyroid glands of the patients treated with strong solution of iodine U. S. P. followed by thiouracil. In these patients subtotal thyroidectomy was performed in two stages. In preparation for the first stage strong solution of iodine U. S. P. only was given. In preparation for the second stage thiouracil was given. Thus the effects of iodine and thiouracil could be compared and contrasted in the same patient. Following the administration of thiouracil, the cells of the acini changed from cuboidal to columnar, and the colloid disappeared or lessened in density and became vacuolated and scalloped.

In the glands removed after the administration of thiouracil followed by strong solution of iodine U. S. P., a refilling of the acini with colloid could be observed, together with a change of the lining cells from columnar to low columnar or cuboidal.

According to these observations, the acinous colloid diminishes in quantity and density or disappears under the influence of thiouracil. The variance between structure and function of the thyroid gland following the administration of thiouracil supports the assertion that thiouracil inhibits the production of new colloid and does not interfere with the use of the available colloid.

The phenomena involved may be understood more easily if one assumes that even under ordinary conditions the acini of the thyroid gland are in various phases of function. These might be called resorptive phase, secretory phase and resting phase.

In the resorptive phase the majority of the acini in each lobule are lined with columnar or low columnar cells and contain light-stained, vacuolated and scalloped colloid. Such is the microscopic appearance of the thyroid gland in untreated patients with hyperthyroidism and in patients treated with thiouracil only.

In the secretory phase the majority of the acini in each lobule are lined with low columnar or cuboidal cells and contain deeper or darker stained homogeneous colloid. Such is the microscopic appearance of the acini of the normal thyroid gland and of the glands of patients with hyperthyroidism treated with strong solution of iodine

3. Halpert, B., and Thuringer, J. M.: Anat. Rec. **91**:16, 1945.

U. S. P. These changes are also seen in the thyroid glands of patients with hyperthyroidism treated with thiouracil followed by strong solution of iodine.

In the resting phase the majority of the acini in each lobule are rather large, are lined with low cuboidal or flat cells and contain deeply or darkly stained homogeneous colloid. This is the microscopic appearance of the acini of thyroid glands with colloid hyperplasia and of the glands of patients with hyperthyroidism treated with strong solution of iodine U. S. P. for a prolonged period.

Thus the effect of thiouracil may be described as intensifying the resorptive phase, and that of strong solution of iodine U. S. P., the secretory phase. Thiouracil and strong solution of iodine U. S. P. administered alternately produce a balance of the functional phases.

SUMMARY

According to these observations, the acinous colloid diminishes in quantity and density or disappears under the influence of thiouracil. The variance between structure and function of the thyroid gland following the administration of thiouracil supports the assertion that thiouracil inhibits the production of new colloid and does not interfere with the use of the available colloid.

SCLEREMA ADIPOSUM NEONATORUM OF BOTH INTERNAL AND EXTERNAL ADIPOSE TISSUE

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CINCINNATI

HARDENING of adipose tissue has been described in medical literature under a wide variety of names and has been attributed to many different causes. When pathologic sections have been taken they have revealed strikingly similar lesions in many of the reported cases even though there has been wide variation in the clinical manifestations. The lesion consists of varying degrees of degeneration, necrosis and sometimes crystallization of adipose tissue together with chronic inflammation more or less granulomatous in nature, often with foreign body giant cells and with a tendency to heal by formation of scar tissue. Most of the reports on this subject make no mention of the state of the perivisceral adipose tissue, and there is often no indication that this tissue was examined microscopically. The pathologic studies of many reported cases have been limited to biopsy material. This limitation and the fact that the cutaneous manifestations of the condition are so striking both clinically and pathologically have led to the generally accepted opinion that such conditions as subcutaneous fat necrosis of the newborn and nonsuppurative panniculitis are confined to the external adipose tissues. This supposition has even been used as a premise by those who assert that the former is caused by obstetric trauma. The case to be described presented widespread involvement of adipose tissue, both internal and external, with lesions which were histologically typical of subcutaneous fat necrosis of the newborn. No other such case was found recorded in the available literature.

REPORT OF A CASE

A Negro infant was born in the Cincinnati General Hospital on Jan. 19, 1943. His 34 year old mother had had three previous pregnancies. These had resulted in the delivery of a full term, 9½ pound (4,309 Gm.) child, now 16 years old, living and well, and two abortions, ten and twelve years later, respectively. A few months before the second abortion she was hospitalized because of pelvic inflammatory disease. One year before the present patient was born, she was found to have diabetes mellitus. She was given insulin and a diet

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which restricted her consumption of butter, milk and fruit. During this fourth pregnancy she had vomited frequently and had lost weight. Nine days before delivery she was admitted to the hospital because of a cyst of one of Skene's glands with urethral stricture. She had not received insulin for two weeks. During fasting the blood sugar was 142 mg. per hundred cubic centimeters. After the diabetes had been brought under control with diet and regular dosage of insulin, labor was induced by rupturing the membranes and releasing about 1,000 cc. of amniotic fluid. Birth occurred twenty-six hours and fifty minutes later. The infant weighed 6 pounds 13½ ounces (3,104 Gm.). He was cyanotic and had paralysis of both arms, that of the left arm being the more marked. He was given 5 per cent dextrose in isotonic solution of sodium chloride subcutaneously and 3.2 mg. of a synthetic preparation of vitamin K (Synkayvite). Oxygen was used to relieve the cyanosis. The next day a pediatric consultant noted petechiae on both cheeks and edema of the skin over the legs and the trunk. On the third day after birth the baby became cyanotic after feeding, and his respirations became irregular. He was transferred to the pediatric service, where he seemed to improve somewhat under routine care during the next few days.

Examined on the ninth day of life, he appeared to be well developed and well nourished. His temperature was 98 F., and his respiratory rate was 20. Hard, nonpitting edema of the subcutaneous tissues was noted in the scalp, the face, the body and the extremities, excluding only the palms, the soles and the genitalia. The facial edema made sucking difficult. There was an ulcer, 2 cm. in diameter, in the scalp over the right occipitoparietal region. There was ulnar deviation of both wrists, and the baby kept his arms flexed at the elbows. Reflexes were absent in the upper extremities and hypoactive in the lower ones, and there was a positive Babinski sign. The heart, the lungs and the abdomen were essentially normal.

On Jan. 21, 1943 the following data were recorded: hemoglobin content, 22 Gm.; red blood cell count, 6,200,000; white cell count, 34,000; differential count, polymorphonuclears 70 per cent, lymphocytes 19 per cent, eosinophils 1 per cent, normoblasts 57 per cent. On this date the blood sugar value was 51 mg. per hundred cubic centimeters. On January 27 the blood sugar values were as follows: one-half hour specimen, 101 mg.; one and one-half hour specimen, 99 mg.; three and one-half hour specimen, 63 mg. The mother's red blood cells were shown to contain an Rh factor. On January 10 a Kahn test of the mother's blood was negative. On February 1 a Kahn test of the baby's blood was negative; the icteric index was 30; the serum protein amounted to 4.38 Gm. per hundred cubic centimeters.

When the blood sugar was found to be low, the baby was given 60 cc. of 5 per cent dextrose in isotonic solution of sodium chloride subcutaneously. For his feeding an evaporated milk formula was adopted (1 : 2 mixture, 3 ounces [88.5 cc.] in six feedings). The baby seemed to improve until the fifth day of life when his respirations again became irregular. He was placed in a Hess bed with oxygen being given as needed. His temperature was around 97 F. most of the time. On the fourteenth day of life a brawny, red, firm, nonpitting induration developed over the back from the waist to the shoulders and extended to the clavicular areas. At the same time the baby was found to have a bilateral bloody nasal discharge. The temperature rose to 101.8 F., the stools became liquid, and the urine was bile stained. The icteric index at this time was 30. The baby was given water by gavage. On the sixteenth day of life he was found dead in his crib.

At autopsy, about twenty-four hours after death, the body weighed 2,975 Gm. and was 48 cm. in length. There was brawny thickening of the skin of the back.

The skin over the rest of the body and the extremities felt firm, as though it were frozen. There was a small ulcer in the right occipitoparietal portion of the scalp. The scleras were slightly icteric. The foreskin of the penis was adherent. There was an abundance of firm adipose tissue beneath the thin, stretched skin. The panniculus varied in color and consistency, but none of it appeared normal. It was all light yellow or white and pasty, in some areas appearing like tallow, and in other places being crumbly and chalky. In some regions, particularly in the left axilla and the left clavicular region and in the perithymic adipose tissue, the fat was streaked with deep pink, watery tissue which appeared to be necrotic. Lymph nodes draining these regions were cherry red, swollen and softer than normal. The subcutaneous tissue of the back was much firmer than normal and seemed to be streaked with fibrous tissue. The right brachial plexus appeared white and glistening, but the left one was dull, pinkish gray and embedded in pink edematous adipose tissue. There was no frank hemorrhage but much congestion in this region. No rupture or other evidence of trauma of the brachial plexus was found, but it was difficult to separate the nerves from the surrounding fat because of interlacing strands of scar tissue. The thymus was swollen, soft and congested. Small subendocardial hemorrhages were scattered over the papillary musculature of the right ventricle. The lungs were subcrepitant and had a few scattered petechial hemorrhages beneath the pleura. The spleen was about one and one-half times the normal size, because of acute congestion. The liver was swollen and slightly icteric. The gastroenteric and genitourinary tracts appeared normal.

Microscopic sections revealed foci of necrosis and beginning acute inflammation, associated with petechial hemorrhages and colonies of short-chained cocci, in various organs and tissues widely disseminated throughout the body. The oldest lesion of this type was the ulcer of the scalp, which appeared to be less than a week old. The baby obviously had terminal septicemia.

The most remarkable type of lesion was found widely disseminated in the adipose tissues of the body. Lesions of this type were frequently found unassociated with the acute septicemic lesions just described, and were definitely much older than any of them. They were characterized by extensive degeneration and necrosis of adipose tissues, with the fats precipitated as needle-shaped crystals, some of which were doubly refractile under the polarizer. The cytoplasm of many of the fat cells stained faintly with hematoxylin and eosin, which is unusual in paraffin sections. Associated with this morphologic evidence of changes in the fat of the adipose tissues was a marked chronic inflammatory reaction with fibrous tissue proliferation and the presence of numerous macrophages, foreign body giant cells and other inflammatory cells. In some regions there was extensive proliferation of fibrous tissue in and around the fat lobules, some of which was hyalinized and appeared to be more than two weeks old. This indicates that the lesion began before the child was born.

The ulcer in the scalp was superimposed on an area of fat necrosis and inflammation. The left brachial plexus was embedded in a similar lesion (fig. 1). Several of the large nerves were surrounded by inflammatory exudate and scar tissue. Lesions in the adipose tissue surrounding the right brachial plexus were of less severity than those on the left side.

At autopsy the only internal adipose tissue which appeared to be abnormal was that around the thymus; this fat seemed to be pinker and firmer than normal. For this reason sections were taken from it for microscopic study. At that time no attempt was made to include fat with the sections of other viscera. On examination of the microscopic sections, the fat of the perithymic region presented not only

acute septicemic lesions but also the typical granulomatous and necrotizing chronic lesions of sclerema adiposum. A careful search of the other routine sections revealed similar lesions in bits of adipose tissue included in one section each of the

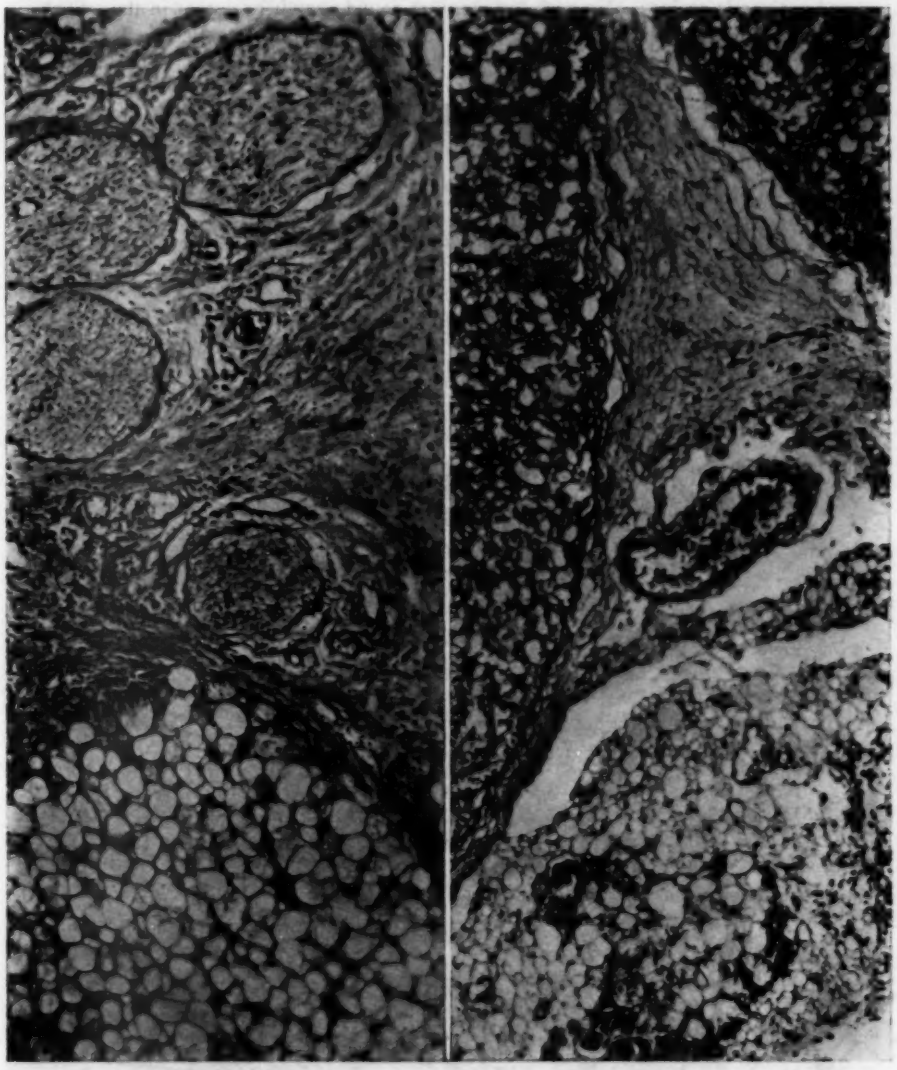

Fig. 1.—Sclerema adiposum around the nerves of the brachial plexus in the left axilla. Hematoxylin and eosin; × 110.

Fig. 2.—Sclerema adiposum in the periadrenal fat. Note the giant cells in the outer margins of the nodules. Hematoxylin and eosin; × 110.

pancreas and the kidney. Fortunately, the infant's body was still in the ice box, since it was one for city disposal. It was still in good condition, although ten days had elapsed since death. Additional samples were taken from adipose tissue in

a wide variety of locations. Microscopic sections of samples of adipose tissue from every location except the scrotum revealed lesions similar in every respect to those found in the panniculus. They included sections from numerous sites near the various viscera, in the mesentery, at the root of the diaphragm and in peribronchial and periaortic regions. No such lesions were found anywhere except in the adipose tissues. The pathologic diagnosis was as follows: terminal septicemia; sclerema

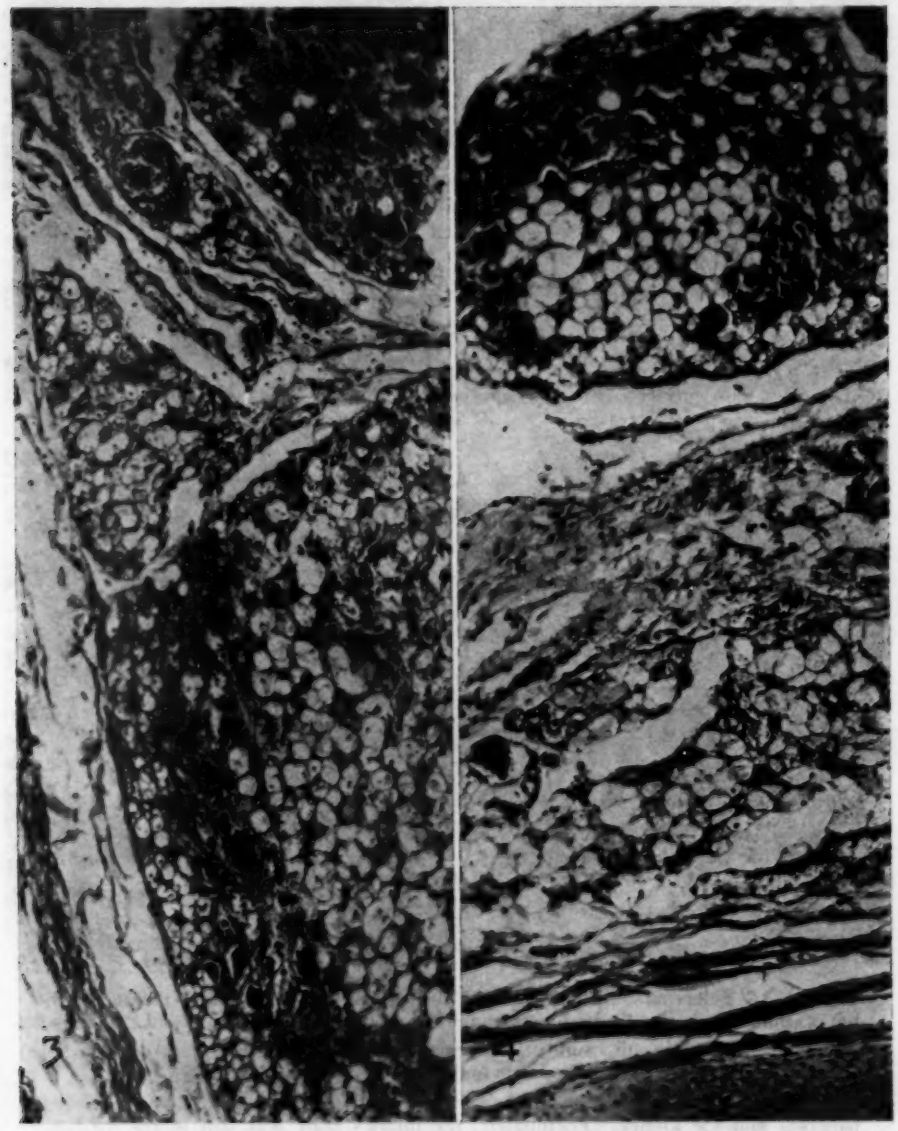

Fig. 3.—Sclerema adiposum in the perithymic fat. Hematoxylin and eosin; × 110.
Fig. 4.—Sclerema adiposum in the peritracheal fat. Hematoxylin and eosin; × 110.

adiposum neonatorum with widespread involvement of subcutaneous and perivisceral adipose tissues; scarring of axillary fat around the left brachial plexus; acute ulceration of a scleremic region in the scalp.

COMMENT

It cannot be stated at present whether this case represents an exception to a general rule concerning the distribution of such lesions of adipose tissues, or whether the internal lesions have been missed heretofore as they were nearly missed in this case. Blocks of infant's viscera taken routinely for microscopic study usually include little adipose tissue. In the cases of adults, pathologic studies of hardening of adipose tissue, such as occurs in febrile nodular nonsuppurative panniculitis and in traumatic fat necrosis of the female breast, have been limited usually to biopsy material. In some of these cases, particularly those of fat necrosis associated with localized trauma caused either by mechanical or by chemical agents, it seems reasonable to suppose that the lesion would remain localized. But in other cases in which there has long been known to be widespread involvement of subcutaneous fat, as in subcutaneous fat necrosis of the newborn and in nonsuppurative panniculitis, such an assumption is unjustifiable until careful search has been made of microscopic sections of the internal adipose tissues in more than just a few cases.

A review of the available literature on nonsuppurative panniculitis revealed only 2 cases in which an autopsy was done during the active stage of this condition. In 1943 Miller and Kritzler [1] reported such a case, in which the examination was limited to "the study of one cutaneous lesion and the organs of the abdomen and chest." The authors found no lesion such as those in the panniculus in the perirenal, mesenteric or pericardial fat, but they saw occasional "lymphocytes, and phagocytes in the epicardial fat." They did not consider the presence of these cells significant. In 1944 Spain and Foley [2] performed autopsy in a case of nonsuppurative panniculitis, in which they found nodules in the mesenteric, omental and pretracheal fat grossly similar to those in the panniculus. However, they thought these lesions were microscopically "dissimilar to the pannicular lesions" and grouped them under "pancreatic fat necrosis" in their diagnosis.

The factors most frequently named as possibly playing a role in the genesis of subcutaneous fat necrosis of the newborn have been reviewed by Fox,[3] Gray [4] and many others. Some of the factors named are as follows:

1. Miller, J. L., and Kritzler, R. A.: Arch. Dermat. & Syph. **47**:82, 1943.
2. Spain, D. M., and Foley, J. M.: Am. J. Path. **20**:783, 1944.
3. Fox, H.: Arch. Dermat. & Syph. **27**:237, 1933.
4. Gray, A. M. H.: Brit. J. Dermat. **45**:498, 1933.

1. Obstetric trauma

2. Low temperature of the body, causing fat to solidify and act as a foreign body

3. Deficiency of olein in the adipose tissue, causing fat to solidify at a higher temperature than is usual for normal fat, possibly at normal body temperature

4. Presence of an abnormal lipolytic ferment

5. Glandular dyscrasia resembling myxedema

6. Varying combinations of the aforenamed factors.

In the present case, although there was a long, somewhat difficult labor, obstetric trauma cannot possibly account for all of the lesions, especially those in well protected internal fat depots. Also, the scar tissue present in some of the fat lobules, particularly that in the region of the left brachial plexus, appeared to be older than the age of the infant. Also, the baby was born with paralysis of the brachial plexus, the only cause found being constriction due to inflammatory reaction and fibrosis associated with the lesions of sclerema adiposum in the surrounding axillary adipose tissue. This baby's temperature ranged around 97 F. most of the time until the onset of the terminal infection; therefore low temperature may have been a factor in accelerating the process after birth.

The most plausible explanation of the lesions in this infant seems to be that they were caused by some generalized disturbance of fat metabolism, the origin of which antedated the birth of the child. The presence of diabetes mellitus and possibly some nutritional deficiency in the mother furnishes interesting ideas, though only for conjecture at present. A review of the reported cases in the literature furnishes proof that not all mothers of such infants have been diabetic.

Chemical studies have been made on subcutaneous tissues by Langer,[5] Smith,[6] Harrison,[7] Chen[8] and others. They furnish substantial evidence that the subcutaneous fat of infants contains a smaller proportion of olein than does that of adults, but opinions vary as to whether there is any essential chemical difference between the fat of normal and that of scleremic infants.

The nomenclature which has been applied to the type of lesion under discussion is in a state of confusion. Most of the names used for this condition have been based on the clinical manifestations of the lesion, and these have presented wide variations. Bailey[9] in reporting 5 cases of

5. Langer, L.: Wien. med. Presse **22**:1375 and 1412, 1881.
6. Smith, C. S.: J. Cutan. Dis. **36**:436, 1918.
7. Harrison, G. A.: Arch. Dis. Childhood **1**:63 and 123, 1926.
8. Chen, T. T.: Nat. M. J. China **16**:360, 1930.
9. Bailey, R. J.: J. A. M. A. **109**:1419, 1937.

nonsuppurative panniculitis lists the following clinical conditions which presumably result from mechanical and chemical damage to adipose tissue and which present similar lesions microscopically: (1) subcutaneous fat necrosis of the newborn, (2) traumatic fat necrosis of the breast, (3) atrophy of fat induced by injection of insulin, (4) paraffinoma, (5) ischemic fat necrosis, (6) lipogranulomatosis, (7) oleogranuloma.

To be added to this list are the numerous terms which Gray [4] found in the literature, applied to the first condition named. After reviewing the long and devious history of the nomenclature of that condition he became a champion of the term "sclerema neonatorum." But this term, which literally means "hardening of the newborn," has been applied to "hardening" conditions in which no change was found in the adipose tissues.

The word "sclerema" comes from the Greek word σκληρός which means "hard." Therefore, "sclerema adiposum" seemed to us to be a good generic term to apply to the lesion in the present case. This term could be applied advantageously to the lesion in all of the conditions just listed, since at some stage of each of them there is hardening of the adipose tissues. Modifying terms could be added to this root term to define further the condition present in a given patient. Examples: "sclerema adiposum neonatorum;" "nodular nonsuppurative sclerema adiposum"; "traumatic sclerema adiposum of the breast." By thus emphasizing the factor common to all of these conditions one might hope to determine sooner their etiology and genesis. Also, the use of this term would help to differentiate those cases in which "hardening" involves adipose tissues from those in which no such lesions are found.

The factor or factors which initiate this lesion are unknown at present. The morphologic aspect of the lesion suggests some disturbance of the nutrition or of the metabolism of fat cells, which so changes the nature of the fat that it acts as a foreign body and thus leads to the characteristic inflammatory reaction and degenerative changes.

Adipose tissue is indeed, as Wells [10] has emphasized, a neglected subject. No longer should it be considered as just so much connective tissue loaded with stored simple fats. Evidence is accumulating to support the concept that the adipose tissue of the body is structurally, developmentally and functionally a distinctive special tissue, more after the order of the ductless glands. The pathologic study of this tissue should not be restricted to an occasional biopsy taken by the dermatologist but should include careful routine examination by the general pathologist.

10. Wells, H. G.: J. A. M. A. **114**:2177 and 2284, 1940.

SUMMARY

In a case of sclerema adiposum neonatorum the lesions were numerous and widely distributed in both the internal and the external adipose tissues of the body. There was evidence that the condition began before birth. The nomenclature of this and other clinical conditions which present similar lesions invites attention, and the suggestion is made that the words "sclerema adiposum," meaning "hardening of adipose tissue," be included in whatever diagnostic term is used pathologically for each of these conditions.

"ALVEOLAR CELL TUMOR" OF THE LUNG

Further Evidence of Its Bronchiolar Origin

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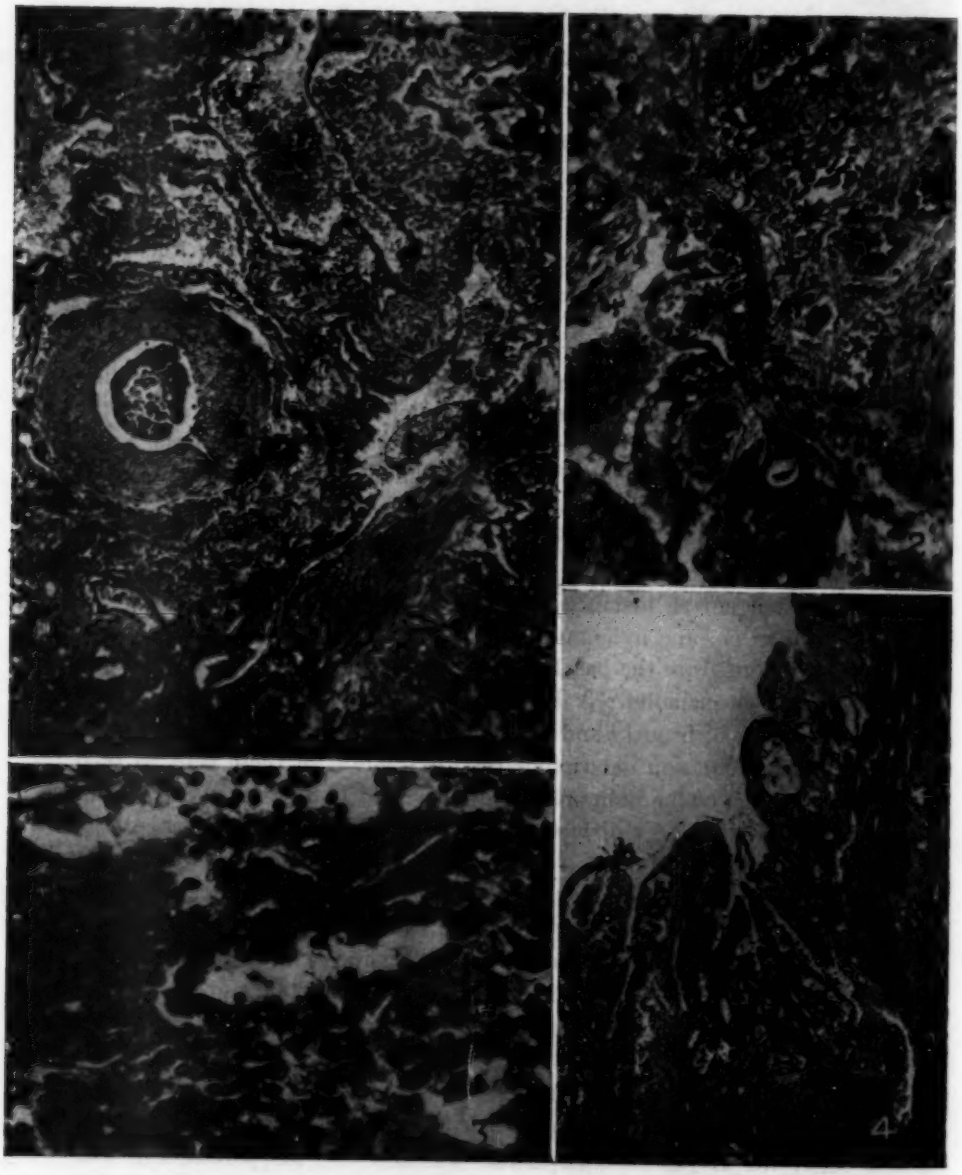
REPORTS of tumors purporting to arise in cells lining pulmonary alveoli continue to appear in medical literature. In a previous communication [1] an attempt was made to analyze available data and determine whether there is any convincing evidence that a primary tumor can arise in this location, or whether the arrangement of tumor cells along the alveolar septums is merely an adaptation of cancer cells arising from the bronchial or the bronchiolar epithelium. From a review of the literature, a study of various chronic inflammatory lesions of the lungs and an analysis of 90 cases of primary pulmonary carcinoma the following conclusions were reached: 1. There is no unanimity of opinion among histologists and embryologists as to whether pulmonary alveoli are lined by epithelial or by mesothelial cells, or indeed by any cells. 2. In most if not all instances the so-called regenerated alveolar epithelium seen in chronic inflammatory lesions of the lungs arises not from septal cells but from the basal cells of the bronchi and particularly from those of the bronchioles. 3. Primary carcinoma of the lung arises from the basal cells of bronchi and bronchioles, and further differentiation or lack of differentiation determines the distribution of these cells in relation to the rest of the pulmonary parenchyma. If they form cuboidal or columnar cells, these will line the alveolar septums in accordance with their inherent property to line spaces and so will produce the well known alveolar distribution. If the cancerous cells form anaplastic or squamous cells, these either will be irregularly distributed throughout the lungs or will occupy the alveolar spaces just as does the exudate in an ordinary type of pneumonia. It was further concluded that since primary bronchiogenic carcinoma is extremely pleomorphic, it rarely is found to be of one uniform cell type and that in a single specimen there are often mixtures of cuboidal, columnar, anaplastic and squamous cells in alveolar, pneumonic or irregular distribution.

After the aforementioned article was accepted for publication, Taft and Nickerson [2] contributed a report of 2 cases of pulmonary mucous

From the Clinical Laboratories, Jefferson Medical College Hospital.

1. Herbut, P. A.: Am. J. Path. **20**:911, 1944.

2. Taft, E. B., and Nickerson, D. A.: Am. J. Path. **20**:395, 1944.



(See legends on opposite page)

epithelial hyperplasia in which they expressed the belief that the hyperplasia originated in the alveolar lining cells but added that an "origin from goblet cells of bronchiolar mucosa cannot be excluded." Subsequently, Ikeda [3] recorded 2 typical cases of alveolar cell tumor and 2 others which were less typical but which nevertheless were designated as such; Wood and Pierson [4] reported 1 case, and Geever, Carter, Neubuerger and Schmidt [5] added 5 cases which had not been previously reported. The latter authors stated that the microscopic examination in all the cases which they presented "favored the belief that the origin of the neoplastic growth was in the alveolar walls. A completely satisfactory proof of this opinion cannot of course be offered."

The question whether primary carcinoma can or cannot arise in cells lining the alveoli is of more than academic interest, for those who contend that such an origin is possible also say that the tumors arise in many foci in both lungs. If this is so, the lesion will be forever hopeless from a therapeutic standpoint. If, on the other hand, the premise that the so-called alveolar cell carcinoma arises from a single focus in a small bronchus or a bronchiole is correct, the prognosis in the future may be even better than that of the more commonly recognized bronchiogenic carcinoma. The latter is frequently so close to the hilus of the lung that complete surgical extirpation is impossible. This is not true of tumors that originate in the more peripheral portions of the bronchial tree, and if a diagnosis is made early, complete removal should be possible. In respect to an early diagnosis some ray of hope is perhaps offered by the current practice of establishing cancer prevention clinics

3. Ikeda, K.: Am. J. Clin. Path. **15**:50, 1945.

4. Wood, D. A., and Pierson, P. H.: Am. Rev. Tuberc. **51**:205, 1945.

5. Geever, E. F.; Carter, H. R.; Neubuerger, K. T., and Schmidt, E. A.: Radiology **44**:319, 1945.

EXPLANATION OF PLATE.

Fig. 1.—A bronchiole lined in part with a single layer of cuboidal cells and in part with a pseudostratified layer of epithelium is shown. The epithelium has extended through the bronchial wall to line adjoining alveolar septums. A blood vessel is filled with tumor cells. Hematoxylin and eosin; × 100.

Fig. 2.—Higher magnification of a portion of the bronchiole illustrated in figure 1 at a different level, showing a lining of cuboidal and pseudostratified epithelium which in the lower portion is penetrating the wall. Note the strands of smooth muscle within the wall of the bronchiole. Hematoxylin and eosin; × 200.

Fig. 3.—An alveolus lined with a single layer of cuboidal cells, three of which are in a state of mitosis. The alveolar septums are greatly broadened and disrupted by extravasated erythrocyes. Hematoxylin and eosin; × 400.

Fig. 4.—Section of the gallbladder showing a zone of transition between the normal epithelium and the cancer cells that line villi and infiltrate the underlying structures in an adenomatous formation. Hematoxylin and eosin; × 25.

where routine periodic roentgenograms of the chest and the finding of cancer cells in bronchial secretions [6] might disclose the lesion while it is still amenable to operation.

It is the purpose of this report to present (1) a very early "alveolar cell tumor" arising in a bronchiole, (2) a primary carcinoma of the gallbladder with metastases in the lungs that grossly and microscopically were indistinguishable from primary "alveolar cell tumor" of the lung, and (3) an analysis of the distribution of metastatic carcinoma of the lungs to see whether, according to cell type, the metastases follow the pattern of primary bronchiogenic neoplasms.

EARLY "ALVEOLAR CELL TUMOR" ARISING IN A BRONCHIOLE

A white woman 69 years old was admitted to the Jefferson Medical College Hospital with a cystocele, a rectocele and prolapse of the uterus. At operation the cystocele and the rectocele were repaired, the uterus was fixed to the anterior abdominal wall and the appendix was removed. Nine days later a sharp pain developed in the lower portion of the right half of the chest, from which she promptly recovered. On the thirteenth day she suddenly became extremely dyspneic and died in shock thirty minutes later.

At necrospy the operative sites were clean. Each internal iliac vein contained a thrombus lying free in the lumen. The main pulmonary artery and each of its branches were filled with a coiled gray embolus similar to the thrombi in the iliac veins. The lateral portion of the lower lobe of the right lung disclosed a deep red infarct 4 cm. in diameter. The lungs were congested and edematous. Nowhere were there tumor nodules. The mediastinal nodes were not enlarged and the remaining organs showed no contributory changes.

Microscopic sections from the site of the infarct of the lower lobe of the right lung disclosed a bronchiole lined in part with a single layer of cuboidal cells and from these a gradual piling up of cells to form a pseudostratified type of epithelium (figs. 1, 2 and 3). At several points the latter had broken through the bronchiolar wall and lined the surrounding alveolar septums with cuboidal or somewhat flattened cells one, two or three layers thick. Although most of the cells were regular, some showed considerable variation in shape and size, as well as hyperchromatism and mitosis. Two blood vessels on each side of the bronchiole were filled with tumor cells similar to those just referred to. Several bands of smooth muscle, which with Van Gieson's stain appeared yellow, were present within the wall of the bronchiole, and throughout the alveolar septums and spaces there was much erythrocytic extravasation. When this lesion was discovered, the right lung was reexamined. No grossly visible tumors were present. Many new sections were then made of the lung in the vicinity of the infarct and between the infarcted area and the hilus. None of the sections from the former site showed tumor, but in one of the sections from the latter site tumor cells were found within a vessel of a larger caliber than those noted in the first sections. Metastases were not found in the lungs or in any of the other organs, and there were no other contributory findings.

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6. Herbut, P. A., and Clerf, L. H.: Bronchiogenic Carcinoma Diagnosed by Cytologic Study of Bronchoscopically Removed Secretions, J. A. M. A., to be published.

CARCINOMA OF THE GALLBLADDER SIMULATING PRIMARY "ALVEOLAR CELL TUMOR"

A white woman 59 years old was admitted to the hospital with a history of griping and colicky pain in the right upper quadrant of the abdomen and constipation of two months' duration. The pain was aggravated by eating, but there was no nausea, and vomiting occurred on only one occasion. Examination disclosed a hard mass 3 cm. in diameter in the region of the gallbladder and generalized tenderness in the right upper quadrant of the abdomen. There were no pulmonary signs or symptoms. Roentgenograms of the chest disclosed enlarged hilar shadows with streaks of density radiating into both lung fields. At operation an irremovable cancer of the gallbladder was found. Five days later she had some indrawing of the sternum and rales in the chest. These persisted, and she died dypneic twelve days after the operation.

Necropsy disclosed slight enlargement of the gallbladder. At its fundus there was a firm, gray, flat tumor that measured 5 cm. across and 1.3 cm. in thickness. It was sharply demarcated from the surrounding mucosa and raised 3 mm. above its surface. Its inner surface was rough and superficially ulcerated, and it penetrated the entire wall to include the serosa. Since most of the tumor was at the tip of the gallbladder and along its inferior border, it did not penetrate the liver substance. The remaining gallbladder wall was several times the normal thickness, firm and indurated, but grossly showed no tumor. The lumen contained thick gray mucoid material and several polygonal black calculi measuring as much as 1.5 cm. in diameter. The cystic duct was stenosed by neoplastic tissue, but the hepatic ducts and the common bile duct were patent. The draining lymph nodes were replaced with mucoid tumor tissue. The liver weighed 1,550 Gm. and contained many circumscribed gray tumors measuring as much as 2 cm. in diameter. There were several cancer nodules in the serosa of the sigmoid colon and one in the left adrenal gland measuring 1.5 cm. in diameter. The peritoneal and each pleural cavity contained 1,500 cc. of straw-colored fluid. The pleural surfaces of each lung were dull but contained no tumors. The pulmonary parenchyma, however, was extensively infiltrated throughout by light gray cancer tissue. Many of the foci were sharply circumscribed and measured 2 to 3 mm. in diameter, while others were more confluent, less sharply defined, and measured 4 cm. across. The centers of many of the larger masses showed beginning necrosis and were filled with mucoid material. The trachea and bronchi were dissected carefully, and no primary tumors were found. The mediastinal lymph nodes were replaced with a gray mucoid cancer tissue, and there were tumor nodules in the diaphragm. The remaining organs showed nothing relevant.

Microscopic sections made of the gallbladder wall at the junction of the tumor with the mocosa showed a rapid transition between the latter and the cancer tissue (fig. 4). The cells became slightly taller and more irregular. Often they were still cuboidal and both covered the gallbladder villi and lined underlying spaces in a single cell layer. Their cytoplasm was pink and abundant and occasionally contained vacuoles. The nuclei were hyperchromatic and varied considerably in size but were usually basilar in position. In some areas the cells were columnar, one to three layers thick and, as before, formed regular acini, while in other areas cuboidal, columnar or irregular cells were loosely arranged, showing no glandular formation. In a few of the sections the tumor was practically confined to the mucosa and the immediately subjacent connective tissue, although in most sections it involved the muscle coats and the serosa. In the

latter there was a marked desmoplastic reaction about the tumor cells. Many sections of the lungs were examined, and all showed cancer cells lining the alveolar septums (fig. 5). The cells were cuboidal or columnar and one or several layers deep. The nuclei were basilar; sometimes they were quite regular, but at other times they varied in shape and size and exhibited considerable hyperchromatism. Mitoses were not seen. Occasionally several nuclei were present within a single cell. The cytoplasm was abundant, pink and at times vacuolated. Papillae projecting into the lumens were abdundant. Usually the cells were closely adherent to the septum, but sometimes they were separated from it. In addition to the peripheral rim of cells, the alveoli occasionally contained tumor in solid masses or in adenomatous formation. In spotty areas they were filled with mucoid material, which was seen to arise from the surrounding epithelial cells. A desmoplastic reaction was rarely seen, but when it was present the entire parenchyma of the lung in these small foci was replaced with

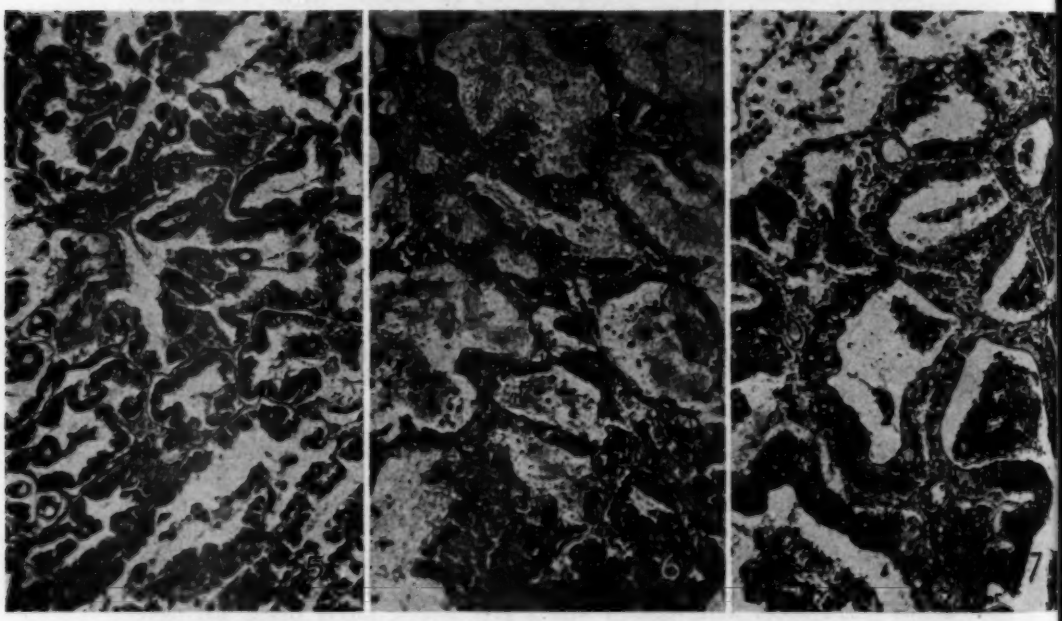

Fig. 5.—Section of the pulmonary metastases of the cancer illustrated in figure 4, showing alveolar septums regularly lined with one to several layers of cuboidal cells. Hematoxylin and eosin; × 100.

Fig. 6.—Section from a tumor of the lung of a patient with mucoid adenocarcinoma of the pancreas, showing some of the alveoli lined with cuboidal cells and all of them filled with mucoid material. Most of those septums which with this magnification appear naked are nevertheless lined with remains of the epithelial cells that produced the secretion. Hematoxylin and eosin; × 100.

Fig. 7.—Section from a tumor of the lung of a patient with adenocarcinoma of the colon, showing pulmonary alveoli regularly lined by one or several layers of columnar cells. There are numerous papillae projecting into the lumens. The lumens also contain some debris. Hematoxylin and eosin; × 100.

fibrous tissue and tumor cells in glandular formation. Cancer cells were found in blood vessels, perivascular lymph channels and bronchioles. In addition to the areas grossly involved with carcinoma, microscopic sections disclosed tumor in the kidneys, the pericardium and lumbar vertebrae.

ANALYSIS OF 125 CASES OF CARCINOMA METASTASIZING TO THE LUNGS

In the last 6,000 consecutive autopsies at the Jefferson Medical College Hospital metastases of carcinoma, exclusive of primary pulmonary neoplasms, were noted in the lungs of 125 persons. The primary sites were in the skin, the sinuses, the breast, the mucous membranes of the mouth, the thyroid gland, the gastrointestinal tract, the liver, the gallbladder, the pancreas, the adrenal gland and the genitourinary system. All histologic sections on file were examined with particular reference to whether the primary and the metastatic tumors had an adenomatous, a solid and compact or an anaplastic and loose arrangement of the cells. The cases were accordingly divided as follows: pure adenocarcinoma, 6 cases; mixture of solid and glandular carcinoma, 14 cases; mixture of anaplastic and glandular carcinoma, 8 cases; solid carcinoma, anaplastic carcinoma or mixtures of the two without adenocarcinoma, 97 cases. There were thus 28 cases in which there was glandular formation, but in only 6 of these was the primary tumor pure adenocarcinoma. These 6 cases were the only ones in which there was produced in the lungs a pure adenomatous formation with alveolar distribution of the cells. Of the remaining 22 cases in this group there were 3 in which the pulmonary metastatic distribution was of the alveolar and pneumonic type, 6 in which it was of the alveolar, pneumonic and irregular type and 13 in which the metastatic foci were of the pneumonic or irregular type or in which the collections of cancer cells were found only in blood vessels, perivascular lymphatic channels or the pleura and immediately subjacent lung. In the 97 cases in which no glandular formation was shown in the primary or the metastatic tumors the arrangement of the metastatic cancer in the lungs was irregular in some, pneumonic in others and in still others a combination of the two.

In the 6 cases in which pulmonary metastases with a pure alveolar distribution were observed, the carcinoma originated in the rectum in 2, in the colon in 2, in the pancreas in 1 and in the gallbladder in 1 (the case described in detail in the foregoing section). The gross distribution of the pulmonary metastases in these cases varied from a single nodule 5 mm. in diameter, in a case of carcinoma of the colon, to a multilobular and coalescing tumor replacing most of the parenchyma of the lung, in the case of carcinoma of the gallbladder. Microscopically (figs. 6 and 7) the cells lined the alveoli in one or several layers, produced papillae projecting into the lumens and were otherwise similar to the cells in the case of primary carcinoma of the gallbladder described in the foregoing section and typical likewise of those in cases of carcinoma described as originating in the pulmonary alveoli.

COMMENT

A bronchiolar or bronchial origin of the tumor with an alveolar distribution has been repeatedly contested on the grounds that such a primary focus has never been demonstrated. I believe it is quite obvious that in the lung any origin in a relatively insignificant structure like a bronchiole would be impossible to find in the mass of cancer tissue always present when a fully developed tumor of this type is examined. In the larger and presumably older nodules, where the tumor probably originated, there are almost invariably foci of necrosis and liquefaction wherein not only the cancer tissue but the entire lung structure is destroyed. For this reason specimens showing the tumor fully developed are of little value in establishing the genesis of this particular tumor, and one must, therefore, turn to a specimen showing the neoplasm in a very early stage, encountered, perhaps, as an incidental finding, as in the first case reported here. In this instance there is no question that the structure lined in part with cuboidal and in part with transitional cells was a bronchiole, for bundles of smooth muscle were seen within its wall both in routine sections and in sections stained with Van Gieson's method. There is likewise no question that the attenuated or cuboidal epithelial cells lining almost all the alveolar septums of several surrounding microscopic fields originated in the bronchiolar epithelium, for in several areas the epithelial cells were seen to penetrate the bronchiolar wall and to be continuous with the alveolar lining cells. The diagnosis of carcinoma was based on (1) the variation in shape, size and staining qualities of some of the cells, (2) the presence of mitoses and (3) the presence of tumor cells within blood vessels.

The second case was described in detail because the lesions of the lungs were grossly and microscopically so typical of a primary "alveolar cell tumor" that had the original site in the gallbladder not been found the case would have been considered as one of primary pulmonary carcinoma. Although in the remaining cases a typical alveolar distribution of the cancer cells was disclosed microscopically, the gross appearance in 3 was less typical of "alveolar cell tumor" in that there were fewer pulmonary nodules and none coalesced or showed lobar consolidation as is sometimes seen in carcinoma primary in the lung. The explanation for this, however, is simple. Unlike the patient with carcinoma of the gallbladder, all 3 patients died from other causes before the pulmonary neoplasms could develop as they otherwise might have. Thus 1 patient died postoperatively from pulmonary embolism, 1 from intestinal obstruction and 1 from obstructive jaundice produced by a metastasis of the tumor in the hilar nodes of the liver. One of the remaining 2 patients who showed widespread pulmonary metastases died of ascites and

peritoneal carcinomatosis and the other of obstructive jaundice produced late in the disease.

A comparison of 125 cases of metastatic pulmonary carcinoma with over 100 cases of primary carcinoma of the lung disclosed that the distribution of the tumors in the lung was the same in each group. Grossly there were a few or many, sharply circumscribed or ill defined, single or confluent nodules, or there were diffuse areas resembling pneumonia irregularly distributed throughout the parenchyma. Microscopically, the distribution of the cancer appeared to depend entirely on the cell type, subject to the peculiarity of the framework of the organ itself. Thus in all tumors, whether primary or secondary in the lung, when the cancer cells were anaplastic and arranged loosely, or when they occurred in solid nests, or when they were in definite squamous cell formation, either they regularly occupied the alveolar spaces in the manner of an exudate in pneumonia or they replaced and entirely obliterated the pulmonary parenchyma. The former arrangement was more prevalent in the tumors unaccompanied by fibrosis, whereas the latter was more often seen in the tumors associated with a desmoplastic reaction. When the primary tumor was pure adenocarcinoma composed of cuboidal and columnar cells, the metatastic cells in the lungs lined the alveolar septums to produce the now often described alveolar cell arrangement. Finally, when the primary neoplasm was composed of a mixture of solid, anaplastic and adenomatous areas, the distribution in the lungs was also mixed but almost always a few cells lined the septums. In both primary and secondary tumors cancer cells were found in blood vessels, in perivascular and peribronchial lymph channels and in the lumens of bronchi and bronchioles.

In view of the foregoing facts it is obvious that any cancer composed of columnar or cuboidal cells in glandular formation will in the lungs line the septums and produce an alveolar arrangement of the cells. Since basal cells of the bronchi and the bronchioles not only are capable of giving rise to columnar or cuboidal cells in acinous formation but have been repeatedly seen to do so in cases in which a bronchial origin for carcinoma has been established, it is concluded that they form the starting point for the primary "alveolar cell tumor" and that there is no justification for assuming that the epithelial cells held to line alveolar septums are capable of giving rise to such a tumor. It is further concluded that in all pulmonary neoplasms showing this distribution of cells the alveolar septums are passive and act merely as a scaffolding on which the cancer cells grow.

SUMMARY AND CONCLUSION

Although reports of carcinoma considered to arise in epithelial cells lining the pulmonary alveoli continue to appear in medical literature,

there is still no satisfactory proof that septal cells are capable of giving rise to primary carcinoma.

In this report I have presented (1) a description of a very early "alveolar cell tumor" arising in a bronchiole and encountered as an incidental finding at autopsy, (2) a detailed description of a primary adenocarcinoma of the gallbladder with the classic gross and histologic alveolar cell distribution in the lungs and (3) an analysis of 125 cases of metastatic pulmonary carcinoma, exclusive of primary cancer of the lung. The macroscopic and microscopic distribution of the neoplasm in these cases of secondary carcinoma followed closely that of primary pulmonary carcinoma, of which over 100 cases have been studied for comparison. In each group when the primary tumor was pure adenocarcinoma the distribution in the lungs was that of an outright alveolar cell arrangement.

In view of these observations it is believed that all tumors presenting an alveolar cell arrangement in the lungs are secondary to recognized or unrecognized foci in other organs, or, if they are primary in the lungs, that they arise from the basal cells of the bronchi and bronchioles and that there is no justification for assuming that they originate in septal cells.

EXPERIMENTAL NEPHROPATHIES

VI. The Problem of Experimental Glomerulonephritis

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AND

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IT IS relatively easy to produce selective, measurable injury of the epithelium of the renal tubules, as has been shown in previous papers in this series.[1] It is much more difficult to injure experimentally the glomeruli alone, or even the glomeruli and the tubules, without involving other organs. Faber [2] expressed the belief that the changes produced in the glomeruli by diphtheria toxin were equivalent "in a general way, to the intracapillary glomerulonephritis of Volhard and Fahr." Bell and Clawson,[3] after repeatedly injecting suspensions of living streptococci into a monkey over a period of four years, expressed the belief that they had "produced a form of chronic diffuse glomerulonephritis which resembles the 'parenchymatous' type of the human disease, but does not correspond to any human lesion in all respects." The nephrotoxic nephritis produced by Pearce,[4] Masugi,[5] Smadel [6] and Sarre [7] is said to resemble or, as reported by some, to be "identical clinically and pathologically" with human nephritis. However, the lack of success of most investigators who have attempted to produce true glomerulonephritis is an indication of the difficulty inherent in the problem.

A study of spontaneous glomerulonephritis in man reveals numerous unit pathologic processes in the glomeruli: circulatory disturbances, such as ischemia, congestion, hemorrhage, thrombosis and embolism; degenerative changes, such as granular degeneration and fatty changes; exudative inflammation, with serous or cellular exudate in the capsular spaces; proliferation of the endothelium; formation of "epithelial crescents" and even complete fibrosis of glomeruli; swelling of the basement

From the Department of Pathology, Northwestern University Medical School.

1. Simonds, J. P., and Hepler, O. E.: Arch. Path. **39**:103 and 133, 1945.
2. Faber, H. K.: J. Exper. Med. **26**:139, 1917.
3. Bell, E. T., and Clawson, B. J.: Am. J. Path. **7**:57, 1931.
4. Pearce, R. M.: Univ. Pennsylvania M. Bull. **16**:217, 1903-1904.
5. Masugi, M.: Beitr. z. path. Anat. u. z. allg. Path. **91**:82, 1933.
6. Smadel, J. E.: J. Exper. Med. **65**:527 and 541, 1937.
7. Sarre, H.: Deutsches Arch. f. klin. Med. **183**:515, 1939.

membrane, and complete necrosis. Some of these changes have been produced in experimental animals. In most cases of human glomerulonephritis, the unitary lesions in the glomeruli are accompanied by changes in the tubules and the interstitial tissue.

In order to account for the genesis of glomerulonephritis it is necessary to explain, first, the diffuse distribution of the lesions. Presumably the injurious agent reaches the kidneys by way of the blood stream. It should, therefore, affect all the glomeruli at the same time and to the same extent, since it reaches all the glomeruli in the same concentration. However, a study of sections of the kidneys involved in this disease in man reveals glomeruli in various stages of involvement; that is, the lesions are of different ages or duration.

The presence of lesions of different ages in the same kidney necessitates the explanation, second, of the temporary escape of some glomeruli from the effects of the injurious agent (that is, the injury of different glomeruli at different times) or of the differences in the rate of progress of the lesions in affected glomeruli. Richards and Schmidt [8] observed the capillaries in a glomerulus of the frog and illustrated changes in caliber extending over a period of forty-five minutes. Hayman and Starr,[9] using Nelson's [10] method, found that the percentage of glomeruli in the rabbit's kidney open to the circulation had a "normal" range between 42 and 100, with that in 10 of the 13 rabbits falling between 56 and 89. Moore and Hellman,[11] using the same method, found that the percentage of open glomeruli in control animals varied from 49 to 89, with an average of 63.8. It is evident, therefore, that there is an intermittency of glomerular circulation in the frog and the rabbit, probably controlled by changes in the arterioles at the point of origin of the capillaries. Whether a similar intermittency occurs in the dog and in man is uncertain, because glomerular activity is more constant in the latter animals than in the rabbit.[12] This intermittency of the glomerular circulation or, in its absence in some animals, "the differential tone of the afferent and efferent arterioles" [12] would protect some glomeruli temporarily from a circulating injurious agent. A colloidal nephrotoxic agent might be removed from the circulation by the reticuloendothelial cells before closed glomeruli became open to blood.

It is necessary to explain, third, the constant or the periodic presence of the injurious agent in the circulating blood. Since the nature and the

8. Richards, A. N., and Schmidt, C. F.: Am. J. Physiol. **71**:178, 1924.
9. Hayman, J. M., Jr., and Starr, I.: J. Exper. Med. **42**:641, 1925.
10. Nelson, B. T.: Anat. Rec. **23**:355, 1922.
11. Moore, R. A., and Hellman, L. M.: J. Exper. Med. **53**:303, 1931.
12. Smith, H. W.: The Physiology of the Kidney, New York, Oxford University Press, 1937, p. 249.

source of this agent are now unknown, it is not possible to account for its apparent irregular entrance into the blood stream.

It would also be desirable to know, fourth, whether the primary glomerular lesion is exudative or proliferative. Unless fibrin is present in appreciable amount and is precipitated in the capsular spaces, the exudative type of lesion usually heals, because the exudate is drained away down the tubules as rapidly as it is formed and does not accumulate to be ultimately organized. The proliferative type of lesion is more likely to end in destruction of the glomerulus involved.

The working hypothesis on which our experiments were based may be stated as follows: (1) A colloidal poison in the circulating blood will not pass through the normal glomerular filter but will be concentrated in its capillaries by loss of water and thus injure these structures; (2) a crystalloidal poison in sufficiently high dilution will pass through the walls of the glomeruli without injuring them into the tubules where, by the absorption of water, it will reach a concentration capable of damaging the tubular epithelium. The second part of this hypothesis served admirably, the evidence for which has been presented in previous papers [1]; work based on the first part was not so successful. We later found that Faber had used the first part of this theory as a basis for his work.

METHOD

The method employed was similar to that described in connection with the effects of inorganic nephrotoxic agents.[1] Our object was to find a dose of a colloidal glomerular poison just sufficient to produce visible injury of the glomeruli without affecting other organs. For this purpose we used snake venoms, the toxin of the diphtheria bacillus and staphylococcus and streptococcus toxins. Sections of the kidneys were stained with hematoxylin and eosin, sudan III and azocarmine and by Gamori's method for phosphatase. Similarly stained sections were made from the kidneys of normal control dogs for comparison.

THE EFFECTS OF SNAKE VENOMS ON THE KIDNEYS

Snake venoms have been used by a number of investigators as nephrotoxic agents. We used the venom of a rattlesnake (Crotalus cinerus) and that of the water moccasin (Agkistrodon piscivorus), both of which act particularly on the endothelium of blood vessels.

Flexner and Noguchi [13] were among the first to study the effects of venoms on experimental animals. They discovered in Crotalus venom an endotheliolytic substance which they called "hemorrhagin." It causes vascular paralysis and capillary hemorrhages due not to simple rupture but to actual disappearance of the walls of the glomeruli as if by solution. Pearce [14] confirmed these observations and noted that when properly graded doses of the venom of Crotalus

13. Flexner, S., and Noguchi, H.: Univ. Pennsylvania M. Bull. **15**:354, 1902-1903.

14. Pearce, R. M.: J. Exper. Med. **11**:532, 1909.

adamanteus were given over a period of one to six days hemorrhage and exudate appeared in the capsular spaces of the glomeruli. In some instances the capillary endothelium seemed to disappear. Eisenbrey,[15] on the other hand, has stated that doses of rattlesnake venom that were large enough to produce renal lesions were so rapidly followed by death that it was not possible to study the functional capacity of the kidneys. With nonfatal doses, no visible renal damage could be identified, and the kidneys of these dogs given these doses excreted from 58.9 to 83.3 per cent of phenolsulfonphthalein. The only evidence of renal injury was a small amount of albumin in the urine. Essex and Markowitz[16] used a rattlesnake venom of such power that 0.4 cc. of a 2 per cent solution would produce in dogs a fall in blood pressure so precipitate as to kill. In such animals they observed, at autopsy, hemorrhages of the medullas and the pelves of the kidneys and of the stomach, the intestines, the adrenal glands and the brain. Milles and co-workers[17] used a rattlesnake venom the minimal lethal dose of which was greater than 0.5 mg. per kilogram of body weight. In the kidneys of dogs poisoned with the venom they observed congestion of the glomerular and intertubular capillaries, with scattered hemorrhages and varying degrees of tubular degeneration. These changes were patchy in distribution, with intervening areas of relatively normal tissue. Taube and Essex[18] used lethal doses of rattlesnake venom that killed dogs in from eight minutes to twenty hours. In dogs that died in a few minutes, the glomeruli were massively congested, and the cells of Bowman's capsule and the tubular epithelium were markedly swollen. In animals that survived for a longer time, the chief changes in the kidneys appear to have been in the proximal convoluted tubular epithelium, which was desquamated, degenerated and necrotic. This change was found "particularly in the subcapsular zone" of the cortex. This indicates that the poison had escaped through the glomerular filter and had affected the first segment of the proximal convoluted tubules, that is, the same part of the renal units that is damaged by minimum necrotizing doses of potassium dichromate.[1] Also, hemorrhage occurred in both the medulla and the cortex. But all other organs, too, were found to be involved.

We injected moccasin venom into 10 dogs and rattlesnake venom into another 10 dogs intravenously. The doses varied from 0.1 to 0.5 mg. per hundred cubic centimeters of blood, and the animals received from one to six doses. Two dogs died in each group. The remaining 16 dogs were killed from one to five days after the first dose. Details of the findings in the dogs whose poisoning was fatal will be related briefly; the findings in the other dogs will be summarized.

Dog KSV 3 died six days after receiving the first of two doses of moccasin venom, each dose being 0.1 mg. per hundred cubic centimeters of blood. During this period the animal lost 19.5 per cent of its body weight. The urine contained albumin (3 plus). The glomeruli were only moderately engorged with blood, the endothelium was proliferated, the basement membrane was swollen, there were fine fat droplets in the hilar region in a few glomeruli, and the capsular spaces contained precipitated albuminous material and regurgitated necrotic tubular epithelium. The epithelium of the proximal convoluted tubules showed marked cloudy swelling and much necrosis that involved groups of cells rather than

15. Eisenbrey, A. B.: J. Exper. Med. **14**:462, 1911.

16. Essex, H. E., and Markowitz, J.: Am. J. Physiol. **92**:317 and 335, 1930.

17. Milles, G.; Müller, E. F., and Petersen, W. F.: Proc. Soc. Exper. Biol. & Med. **28**:561, 1931.

18. Taube, H. N., and Essex, H. E.: Arch. Path. **24**:43, 1937.

entire segments of the tubules. The phosphatase appeared normal in amount and distribution except that it had diffused into the cytoplasm of the necrotic cells. The liver and the adrenal glands showed striking congestion and some necrosis.

Dog KSV 18 died two days after receiving the first of two doses of moccasin venom, each dose being 0.5 mg. per hundred cubic centimeters of blood. This animal lost 10.35 per cent of its body weight. No albumin was demonstrated in the urine. The glomeruli were moderately congested, and many nuclei were pyknotic. The basement membrane was swollen, and the capsular spaces contained precipitate. The epithelium of the proximal convoluted tubules showed cloudy swelling and a moderate amount of necrosis. The phosphatase appeared to be normal in amount and distribution. The liver was severely congested with much central necrosis. The walls of many central veins were infiltrated with polymorphonuclear leukocytes and monocytes. Some of the hepatic cells were of giant size. There was extensive hemorrhage in the mucosa of the intestines and in the alveoli of the lungs.

Dog KSV 2 received 4 doses of rattlesnake venom, 2 doses were 0.1 mg. each, and 2 doses were 0.2 mg. each, per hundred cubic centimeters of blood. The animal died five days after the first dose. It had lost 27.6 per cent of its body weight. The urine was red (hemoglobin). The glomeruli were only moderately congested; many of their nuclei were pyknotic, and some were disintegrating; the basement membrane was swollen; many glomeruli contained considerable quantities of fat, and precipitate and regurgitated necrotic tubular epithelium were present in many capsular spaces. The epithelium of the first segment of the proximal convoluted tubules contained a good deal of fat and showed marked cloudy swelling and much necrosis, with red debris in the lumens. The phosphatase appeared to be somewhat reduced in amount and was diffused into the cytoplasm. The liver was fatty and markedly congested, and the central cells were necrotic. The adrenal glands were also congested and showed much necrosis.

Dog KSV 17 died twenty minutes after receiving one injection of rattlesnake venom, the dose being 0.5 mg. per hundred cubic centimeters of blood. The glomeruli were markedly congested and had some precipitate in the capsular spaces. The convoluted tubules appeared unchanged. The liver and the spleen were massively congested, and the red blood cells appeared to be clumped.

Lesions resulting from the action of the venoms in these 4 dogs were not limited to the kidneys. The liver and the adrenal glands were the organs most severely and constantly damaged. In the kidneys both glomeruli and tubules were injured.

The findings among the other 8 dogs poisoned with moccasin venom may be summarized as follows: Two of 5 dogs had albumin in the urine. Four of 5 were shown by the benzidine test to have blood (hemoglobin) in the urine. The renal phosphatase appeared to be normal in amount and distribution. Only 1 dog had easily visible fat in the glomeruli. In 3 dogs the glomeruli were essentially unchanged; in the others there was moderate congestion with varying degrees of endothelial proliferation. The epithelium of the proximal convoluted tubules of all dogs showed cloudy swelling, but in only 1 was there any noteworthy degree of necrosis. The loss of body weight in these 8 dogs varied from 0 to 12.60 per cent. In 3 of the 8 dogs other organs were involved as follows:

Dog KSV 5 received two doses of moccasin venom, each dose being 0.2 mg. per hundred cubic centimeters of blood, and was killed four days after the first dose. The liver and the spleen were definitely congested; there was some necrosis in the liver. The adrenal glands were unchanged.

Dog KSV 13 was given two doses of moccasin venom on the same day, each dose being 0.4 mg. per hundred cubic centimeters of blood, and was killed two days thereafter. This animal had hemorrhages in the mucosa of the stomach, in the interstitial tissue of the pancreas and in the triads of the liver.

Dog KSV 19 received two doses of moccasin venom, each dose being 0.5 mg. per hundred cubic centimeters of blood, and was killed two days later. There was hemorrhage in, and destruction of, many islets of the pancreas. The liver and the spleen were markedly congested, with areas of necrosis. In the liver the red blood cells appeared to be fused into compact masses. A lymph node was hemorrhagic, and there was pronounced phagocytosis of red blood cells in the sinusoids.

The lesions in the 8 dogs receiving rattlesnake venom may be summarized as follows: Two of 7 had albumin in the urine, and 2 of 4 were shown by the benzidine test to have blood (hemoglobin) in the urine. The renal phosphatase was normal in amount and distribution. Three dogs had easily visible fat in the glomeruli. In 2 dogs the glomeruli were definitely congested; in 3 there was hemorrhage and precipitate, and in 1 there was regurgitated necrotic epithelium in the capsular spaces. Endothelial proliferation was not marked. In all dogs in this group the epithelium of the proximal convoluted tubules showed cloudy swelling, but in only 2 was necrosis present in noteworthy amount. One dog had red blood cells in some of the tubules. The loss in body weight ranged from less than 1 to 9.75 per cent. Only 1 dog in this group of 8 showed noteworthy lesions in other organs. Dog KSV 6 received two doses of venom, each dose being 0.2 mg. per hundred cubic centimeters of blood, and was killed four days after the first dose. The liver and the spleen were congested, and the liver showed areas of necrosis. The adrenal glands were apparently normal.

The loss of weight of these dogs poisoned with snake venoms varied with the dose and the period of survival of the animal. Among those poisoned with moccasin venom, the greatest loss of weight was 19.5 per cent in six days. In this group: 6 lost from 2.16 to 19.5 per cent of their original body weight; 2 gained from 3.96 to 5.55 per cent; in 2 the weight remained approximately the same as at the beginning of the experiment. The greatest loss of weight among the dogs poisoned with rattlesnake venom was 27.6 per cent in five days. Nine of the 10 dogs in this group lost from 0.7 to 27.6 per cent, while the tenth animal died within twenty minutes after the administration of the venom. This loss of weight is an indication that the poison has far reaching effects on the entire body of the animal.

Four of 5 tested among the dogs poisoned with moccasin venom and 2 of 4 tested among those poisoned with rattlesnake venom were shown by the benzidine to have blood (hemoglobin) in the urine. This finding was probably the result of the known hemolytic effect of these two types of venom. The smallest dose of venom of each type to produce hemoglobinuria was 0.2 mg. per hundred cubic centimeters of blood.

Simonds and Brandes[19] found that the mean kidney weight–body weight ratio of normal dogs was 0.0057. The ratios for the 2 dogs that died after poisoning with moccasin venom were 0.0062 and 0.0078, respectively, with an average of 0.0070. The ratios for the other 8 dogs, in which the poison was not fatal during the period of the experiment, ranged from 0.0042 to 0.0058, with an average of 0.0047. The average ratio for the entire group of 10 dogs was 0.0052. The kidney weight–body weight ratios of the 2 dogs fatally poisoned with rattlesnake venom were 0.0037 and 0.0061, average 0.0049. The ratios of the other 8 dogs in this group ranged from 0.0047 to 0.0069, average 0.0055. The average

19. Simonds, J. P., and Brandes, W. W.: Arch. Path. 9:445, 1930.

for the entire group of 10 dogs was 0.0054. The cause of the marked relative increase in the weights of the kidneys of the dogs fatally poisoned with moccasin venom was apparently the accompanying high degree of cloudy swelling and necrosis of the proximal convoluted tubules, since the kidneys of these animals were not seriously congested. The relative increase in the weights of the kidneys of the dog that died withi ntwenty minutes after injection of rattlesnake venom was due to the extreme degree of congestion of these organs.

The mean liver weight–body weight ratio of normal dogs was found by Simonds and Brandes [19] to be 0.0303. The ratios for the 2 dogs fatally poisoned with moccasin venom were 0.0329 and 0.0520, respectively, average 0.0425; the ratios for the other 8 dogs ranged from 0.0210 to 0.0332, average 0.0267. The average ratio for the entire group of 10 dogs was 0.0310. The liver weight–body weight ratios of the 2 dogs that died after poisoning with rattlesnake venom were 0.0269 and 0.0294, average 0.0282. Among the other 8 dogs in this group the ratios ranged from 0.0239 to 0.0341, average 0.0286. The average ratio for this entire group of 10 dogs was 0.0285. These ratios indicate that moccasin venom, particularly, produced a relative increase in the weight of the liver as a result of the accompanying severe congestion and necrosis.

THE EFFECTS OF DIPHTHERIA TOXIN ON THE KIDNEYS

Diphtheria toxin has been a favorite nephrotoxic agent, used by numerous investigators. Its effects vary with the dose and the time of survival of the animal. Lyon [20] used a toxin of such potency that 0.007 cc. killed rabbits in three to five days. Doses of 0.004 to 0.006 cc. might kill the animal in twelve days, while rabbits given 0.003 cc. usually survived but always had albumin and casts in the urine. Karsner and Denis [21] gave cats daily injections of 0.1, 0.25 and 0.5 unit of diphtheria toxin for periods up to twenty-seven days. With the smallest dose albuminuria was present after the seventh day; with larger doses it appeared earlier. Faber [2] thought that the lesions produced by diphtheria toxin corresponded in a general way to the intracapillary glomerulonephritis of Volhard and Fahr. With properly graded doses it is usually possible to injure the kidneys of an animal without producing visible effects in other organs. Diphtheria toxin injected intravenously disappears quickly from the blood. Bieling and Gottschalk [22] found more than half of the injected toxin in the abdominal organs, particularly in the spleen. Speransky [23] asserted that shortly after introduction into the blood stream a small amount of diphtheria toxin is excreted unchanged by the kidneys.

Human patients with diphtheria frequently have albumin and casts in their urine, and various lesions are found in their kidneys. Earlier investigators (Cornil and Brault [24]; Langhans [25]; Oertel [26]) record damage of the tubular epithelium as the most frequent and severe lesion found in the kidneys of patients dead from diphtheria. The predominant lesion in 117 of 171 cases of fatal

20. Lyon, G.: J. Path. & Bact. **9**:400, 1904.

21. Karsner, H. T., and Denis, W.: J. M. Research **19**:270, 1914.

22. Bieling, R., and Gottschalk, A.: Ztschr. f. Hyg. **99**:125 and 142, 1923.

23. Speransky, A.: Ann. Inst. Pasteur **41**:1063, 1927.

24. Cornil, A. V., and Brault, A.: Études sur la pathologie du rein, Paris, F. Alcan, 1884, p. 149.

25. Langhans, T.: Virchows Arch. f. path. Anat. **99**:193, 1885.

26. Oertel, M. J.: Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung, Leipzig, F. C. W. Vogel, 1887.

diphtheria studied by Councilman, Mallory and Pearce [27] was that of the tubules; in 43 cases there was interstitial nephritis characterized by infiltration of the intertubular tissues with plasma cells and lymphocytes; in the remaining 11 cases acute glomerulonephritis was the chief lesion. One of us [28] studied 7 cases of fatal diphtheria and found severe damage of both glomeruli and tubules in all cases. It is evident from this brief review of the literature that in man diphtheria toxin absorbed from the primary site of the disease injures predominantly the tubular epithelium. This may be due in part to the greater ease with which injury of the tubules can be detected and in part to the relatively small size of the colloidal molecule of the toxin, which allows it to escape through glomeruli that have been only slightly damaged by this agent.

Numerous pathologic processes observed in the glomeruli of experimental animals after injection of diphtheria toxin have been described. They include: congestion (Lyon [20]; Faber [2]); thrombosis, often hyaline in nature (Karsner and Denis [21]; Bailey [29]; Lyon [20]; Frothingham [30]); increase in polymorphonuclear leukocytes (Flexner [31]; Lyon [20]; Bailey [29]; Schlayer and Hedinger [32]; Takayasu [33]); proliferation of the endothelium of the glomerular capillaries (Faber [2]; Karsner and Denis [21]; Schlayer and Hedinger [32]; Takayasu [33]); swelling and degeneration of the glomerular epithelium (Lyon [20]); hyaline degeneration (Lyon [20]); fatty degeneration (Bailey [29]; Lange [28]); precipitation in the capsular space (Faber [2]; Karsner and Denis [21]; Schlayer and Hedinger [32]; Takayasu [33]); necrosis of the glomeruli (Flexner [31]; Faber [2]; Bailey [29]); mitotic activity in the glomerular endothelium (Flexner [31]); formation of "epithelial crescents" (Takayasu [33]).

A smaller number of pathologic processes have been described in the tubules: fatty degeneration (Bailey [29]; Lyon,[20] who calls attention to the necessity of using an animal which does not normally have fat in its tubular epithelium); cloudy swelling (Lyon [20]; Karsner and Denis [21]; Schlayer and Hedinger [32]; and others); necrosis of the tubular epithelium (Karsner and Denis [21] and others observed this change chiefly in the proximal convoluted tubules, while Lyon [20] found it most marked in the ascending limb of Henle's loop); deposition of granular debris in the lumens of the tubules (Lyon,[20] and others) casts (Lyon [20]; Bailey,[29] and others), and sometimes blood casts. Calcification is mentioned only by Flexner.[31]

In our experiments we injected diphtheria toxin intravenously into 21 dogs, the doses being equivalent to 0.6 and 0.8 minimum lethal dose (M.L.D.) per hundred cubic centimeters of blood. Two animals, because of a mistake in calculation, were given six times the minimum lethal dose. The dogs were given one to eight doses and were killed from one to four days after the first dose. Some of the animals were therefore given as many as three injections in one day.

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27. Councilman, W. T.; Mallory, F. B., and Pearce, R. M.: J. Boston Soc. M. Sc. **5**:139, 1900-1901.

28. Lange, J.: A Study of the Effects of Diphtheria Toxin (An Example of a Colloidal Poison) on the Kidney, Thesis, Northwestern University Graduate School, 1942.

29. Bailey, C. H.: J. Exper. Med. **25**:109, 1917.

30. Frothingham, C.: J. M. Research **30**:365, 1914.

31. Flexner, S.: Bull. Johns Hopkins Hosp. **6**:259, 1897.

32. Schlayer and Hedinger: Deutsches Arch. f. klin. Med. **90**:1, 1907.

33. Takayasu, H.: Deutsches Arch. f. klin. Med. **92**:127, 1908.

Congestion of the glomeruli was striking and widely diffuse in some dogs; in others it was more irregular, involving only some of the glomeruli or even parts of the glomeruli. Many glomeruli were large, completely filling the capsular spaces. In some this was due to congestion; in others, to proliferation of the endothelium of the glomerular capillaries. The glomeruli that had the greatest amount of endothelial proliferation showed the least amount of congestion. In 1 dog (KDph 2) that received six times the minimum lethal dose the glomeruli were small because they were compressed by the large amount of material in the capsular spaces (fig. 1). In most of the glomeruli the basement membrane was swollen. The degree of swelling appeared to be proportional to the size of

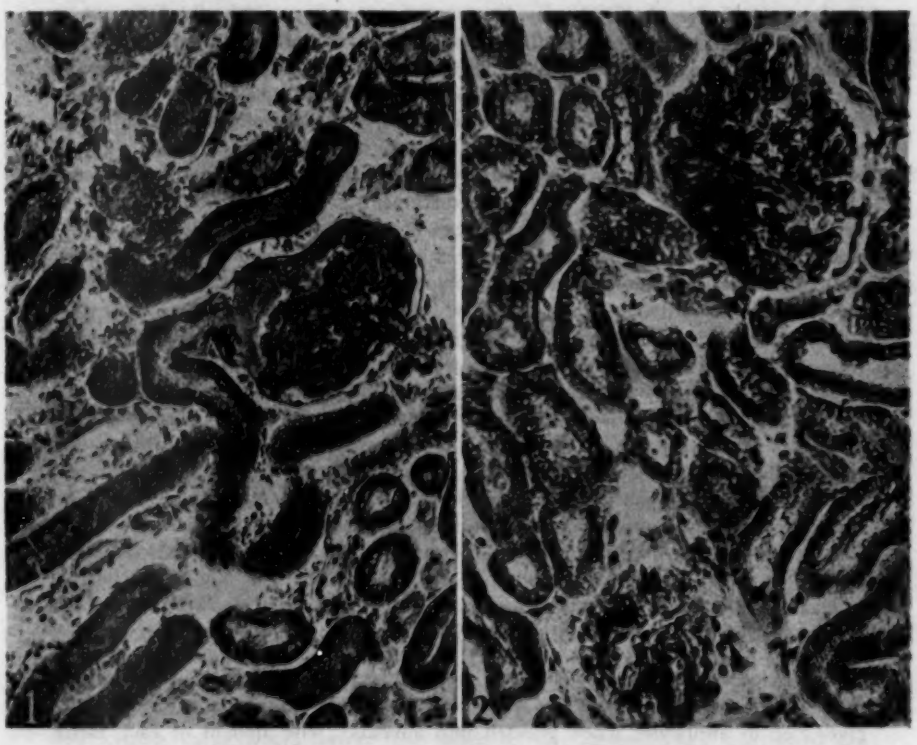

Fig. 1.—Section of kidney from dog KDph 2, which received six times the minimum lethel dose of diphtheria toxin per hundred cubic centimeters of blood and died on the third day thereafter. The capsular space is filled with precipitated protein. The nuclei of the glomeruli and of the tubules are pyknotic. The tubular epithelium is swollen and granular, and some cells are necrotic. × 155.

Fig. 2.—Section of kidney from dog KDph 3, which received 0.6 M.L.D. of diphtheria toxin per hundred cubic centimeters of blood and was killed forty-eight hours later. Most of the glomeruli contained much fat (black in the photomicrograph). × 155.

the dose, although in only 2 of the 9 instances in which the dose was 0.8 M.L.D. were sections of the kidneys stained with azocarmine. Fat in quite large amounts was seen in the glomeruli of 5 dogs (fig. 2). The proportion was higher among those receiving 0.8 M.L.D. The albuminous material precipitated in the capsular spaces was scanty except in 1 dog that received one dose six times

the minimum lethal dose and died four days later. In this animal practically every capsular space was filled with this material. In many glomeruli the nuclei were quite pyknotic. Small blue-black masses resembling chromatin liberated from nuclei by karyorrhexis were also present in many glomeruli. These were more numerous in the kidneys of dogs given the larger doses of toxin. In these dogs there were hemorrhages in the gastric mucosa, the serous membranes and the adrenal glands and central necrosis in the liver.

In all the dogs of this group the epithelium of the proximal convoluted tubules showed cloudy swelling. In some animals the swelling was so marked that the lumen was almost obliterated. In others, the lumens contained much granular debris, but no definite casts were observed. Fatty changes were observed in the part of the proximal convoluted tubules that lay within the labyrinth in 10 dogs. This is probably significant, since fat is not present in this portion of the proximal convoluted tubules in normal dogs. Its presence in the terminal portion of these tubules has no pathologic significance.[1] Necrosis was found in many proximal convoluted tubules. It tended to involve single cells or groups of cells rather than entire segments of tubules as in the kidneys of dogs poisoned with crystalloidal, inorganic agents. It did not have any characteristic location but appeared to occur chiefly in the upper segments. We did not observe necrosis in Henle's loop.[20] Sections of the kidneys of 11 dogs were stained by Gomori's method. In general, phosphatase was present in normal amount and distribution. In a few instances there was a slight tendency to diffuse into the cytoplasm of the cells.

Albuminuria was not constantly observed. Only 10 of the 20 dogs examined were found to have albumin in their urine. Casts were present in a slightly larger number, namely 14. The kidney weight–body weight and liver weight–body weight ratios increased with the size of the dose. This indicates a greater degree of injury of the kidneys with increase in the size of the dose. The relative increase in the weights of the kidneys and the liver was due in part to congestion and in part to cloudy swelling and necrosis. No changes of significance were observed in the interstitial tissues. In a few dogs there was some congestion of the intertubular capillaries. But no distinct alterations could be seen in these structures.

THE EFFECTS OF STAPHYLOCOCCUS TOXIN ON THE KIDNEYS

Staphylococcus aureus produces a toxin that has leukotoxic, hemolytic and tissue-necrotizing[34] effects. The three components are protein in nature, as each gives rise to antibodies when injected into animals. The amount of each factor varies in the toxins of different strains of staphylococci. Julianelle[35] stated that all pathogenic strains produce hemolysin. The tissue-necrotizing toxin has been studied by Gross,[36] by Burnet[37] and by Bigger and his co-workers.[38]

A dose of staphylococcus toxin can be selected that will limit its effects to the kidneys without producing visible injury in other organs of the body. It should be a toxin in which the necrotizing factor exceeds the leukotoxic and

34. Gay, F. P.: Agents of Disease and Host Resistance, Springfield, Ill., Charles C Thomas, Publisher, 1935.

35. Julianelle, L. A.: J. Infect. Dis. **31**:256, 1922.

36. Gross, H.: Centralbl. f. Bakt. (Abt. 1) **115**:367, 1930.

37. Burnet, F. M.: J. Path. & Bact. **32**:717, 1929; **33**:1, 1930; **34**:471, 1931.

38. Bigger, J. W.; Bland, C., and O'Meara, R. A.: J. Path. & Bact. **30**:261, 1927.

hemolytic factors. It is more difficult to select a dose that will act only on the glomeruli without producing extensive injury of the tubular epithelium. Von Glahn and Weld [39] used rabbits and cats and concluded that staphylococcus toxin damages the kidneys selectively and that its primary site of action is the glomerular capillaries and other small blood vessels. The extent of the damage is proportional to the amount of toxin administered. They described the following changes occurring in the kidneys after poisoning with staphylococcus toxin: 1. The earliest visible change is an extraordinary distention of the glomerular capillaries. 2. With more extensive damage, the endothelial lining of the glomerular capillaries disappears, and a layer of fibrin covers the inner surfaces of the capillary loops. 3. A precipitate of albuminous material is formed in the capsular spaces although these spaces are almost completely filled with the extremely congested glomeruli. 4. The congestion extends from the glomeruli backward along the course of their blood supply through the afferent arteries to the intralobular arteries, frequently with complete necrosis of the walls of the arteries. 5. Accompanying these changes in the glomeruli and blood vessels, changes in the epithelium of the proximal convoluted tubules range from cloudy swelling and fine granulation of the cytoplasm to necrosis that may be so extensive as to involve the greater part of the cortex.

Rigdon and his co-workers [40] agreed with Von Glahn and Weld as to the essential details of the renal effects of this toxin and add that extensive thrombosis accompanies the damage to the glomerular capillaries and small arteries. Rigdon [41] used dogs and rabbits and concluded that the renal effects of staphylococcus toxin may be conveniently divided into two groups, namely, hemorrhagic and necrotic. The hemorrhagic type was more frequently encountered in dogs and the necrotic type in rabbits. However, he observed that the lesions are influenced by the quantity of toxin, the method of administration and the length of time elapsing before death. The hemorrhagic and necrotic areas suggested that the toxin either injures the endothelium of the capillaries, causing thrombosis and infarction, or injures the epithelial cells by acting directly on these cells. The dog appears to be more susceptible to the action of this toxin than does the rabbit, hence it is possible that the differences in the types of lesions found in the kidneys of the dog may be due to the earlier death of this animal. Rigdon and his associates [40] were unable to demonstrate a sufficient number of thrombi to consider the necrosis secondary to obstruction of blood vessels. They concluded, therefore, the toxin acts directly on the tubular epithelium and is the chief cause of the extensive necrosis observed.

Massive cortical necrosis as a result of the action of staphylococcus toxin was known many years before the work of the investigators quoted in the preceding paragraph. According to Neisser and Levaditi [42] and Neisser and Wechsberg,[43] the necrosis is equivalent to infarction. Rigdon and his co-workers [40] commented on the difficulty of differentiating quantitatively between the necrosis which is the result of the circulatory disturbances and that which is due to the direct action of the toxin when it comes in contact with the tubular epithelium

39. Von Glahn, W. C., and Weld, J. T.: J. Exper. Med. **61**:1, 1935.

40. Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: Am. J. Path. **10**:425, 1934.

41. Rigdon, R. G.: Arch. Path. **20**:201, 1935; Arch. Int. Med. **57**:117, 1936.

42. Neisser, M., and Levaditi, C.: Compt. rend. Cong. internat. de méd. (Sect. de path. gén. et de path. expér.), 1900, p. 475.

43. Neisser, M., and Wechsberg, F.: Ztschr. f. Hyg. u. Infektionskr. **36**:299, 1901.

after it has escaped through the damaged glomerular filter into the lumens of the tubules. Rigdon [41] concluded that both factors are concerned in the process of necrosis. He also studied the lesions in the kidneys of 7 patients who died from staphylococcic infections and noted in the kidneys of some of them changes which resembled those observed in experimental animals.

In our experiments we attempted to find a dose of staphylococcus toxin that would produce minimal lesions in the kidneys of dogs without causing the extensive hemorrhages and the massive necrosis observed by Rigdon [41] and others. Twelve dogs were given multiple (2 or 3) daily intravenous doses of the toxin, the doses being 0.06 and 0.07 cc. per hundred cubic centimeters of blood. Altogether, from two to seven doses were administered, and the animals were killed in from one to four days after the first dose. These doses affected both glomeruli and tubules but did not induce extensive hemorrhages or massive necrosis.

Since we used only small doses, the changes differed from those reported by others in the extent, rather than in the character, of the lesions, and it is not necessary to describe them in detail. The most constant changes observed in our dogs were marked congestion of the capillaries and swelling of the basement membrane of the glomeruli and cloudy swelling and necrosis of the epithelium of the proximal convoluted tubules. Although the capillaries of the glomeruli were excessively distended with blood, the amount of precipitate in the capsular spaces was minimal, and no red blood cells were seen either in the capsular spaces or in the lumens of the tubules. Definite, but only occasional, thrombi were found in the glomerular capillaries. These were observed more frequently in the dogs that received the larger doses, but there was no constant relation to the number of doses. The basement membrane of the glomeruli was swollen in all 7 dogs sections of whose kidneys were stained with azocarmine. The swelling and irregularity of the membrane were more pronounced in dogs that received the larger doses. In most of these dogs there was definite, but often irregular, proliferation of the endothelium of the glomerular capillaries. The proliferation was most marked in the region of the hilus of the glomerulus involved. It was often limited to a single loop. In some glomeruli there were one or more huge, moderately pyknotic oval or indented nuclei. Disintegrating nuclei appeared as one to several dark blue granules. Polymorphonuclear leukocytes were not noticeably increased in the glomeruli above the number that would be expected in the increased quantity of blood. In 2 dogs fat in considerable amount was visible in many glomeruli.

In all dogs, regardless of the size or the number of the doses, the epithelium of the proximal convoluted tubules showed marked cloudy swelling. In some tubules the epithelium was merely swollen and the lumen almost obliterated. In others the inner margins of the cells were irregular and ragged, while the lumens were fairly large and contained granular material. No definite casts were observed in any of the tubules, although in some the material in the lumens was compact and granular. Some degree of necrosis of the epithelium of the proximal convoluted tubules was observed in all dogs. The necrosis rarely involved all the cells in any segment of a tubule. Rather, it involved groups of cells. The appearance of the necrotic cells varied. Some were entirely without visible nuclei; in others the nuclei were visible but pale and shadowy; in still others the nuclei were broken up into fragments or appeared as a single, small, compact mass of blue-black chromatin. The necrosis was sometimes present in, or entirely

limited to, the zone between the renal capsule and the first row of glomeruli. This indicates that the toxin was present in sufficient strength to affect the cells in the first segment of the proximal convoluted tubules.

THE EFFECTS OF STREPTOCOCCUS TOXIN ON THE KIDNEYS

The frequency with which the kidneys are involved in scarlet fever, erysipelas and other streptococcic infections, particularly those of the upper respiratory tract,[44] led us to attempt to injure the glomeruli with streptococcus toxin. Mallory and Keefer [45] found the kidneys involved in 24 (54 per cent) of 44 cases of scarlet fever and in 8 (36 per cent) of 22 cases of fatal erysipelas. But the lesions appear to have been entirely of the interstitial type, characterized by presence of lymphocytes and plasma cells between the tubules and about the glomeruli, as originally described by Councilman.[46] Brody and Smith [47] found lesions in the kidneys in 32 (72.6 per cent) of 44 cases of scarlet fever. In only 1 of these cases was typical glomerulitis shown; in the remainder the involvement was of the interstitial type. In 8 other cases "some histologic suggestion of very early glomerular changes was seen." They commented that perhaps a partial explanation of the comparative rarity of the glomerular type of lesion is that more than three quarters of their patients died before the twenty-first day of their illness, before the glomerular type of nephritis ordinarily appears.

Living and killed streptococci and various products derived from these organisms have been used by different investigators in attempts to injure the glomeruli. Bell, Clawson and Hartzell [48] injected live cultures of "streptococci from human lesions" into rabbits and monkeys. In the rabbits they found only "the ordinary interstitial type, i. e., lymphocytic interstitial nephritis." With 14 monkeys used, severe renal lesions were produced in only 5: nephrosis in 2; acute interstitial nephritis in 1, and acute glomerulonephritis in 1; the fifth monkey was killed after four years of repeated injections of living streptococci and was found to have "chronic diffuse glomerulonephritis which resembled the human disease but not in all respects." [3] Stoddard and Woods [49] injected powdered washed streptococci into rabbits and produced vacuolar degeneration of the renal tubules, giving the cells the appearance of fatty changes, up to the collecting tubules. Lukens and Longcope,[50] using rabbits and injecting heat-killed streptococci directly into one renal artery, produced glomerulonephritis, sometimes focal, sometimes diffuse.

Brody and Smith [47] expressed the belief that the evidence presented by them suggests that the lesions which they described were the result of the circulation of a toxin. Mallory and Keefer,[45] on the other hand, held that the late tissue reactions observed in streptococcic infections are the response of the host to the infection and that they are the result in part of an antigen-antibody reaction rather than of direct toxic damage of the tissues. The fact that nephritis usually develops only after the acute symptoms of scarlet fever have subsided suggests that the lesions in the kidneys are related to an antigen-antibody reaction. The

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44. Seegal, D.; Seegal, B. C., and Lyttle, J. D.: J.A.M.A. **105**:17, 1935.
45. Mallory, G. K., and Keefer, C. S.: Arch. Path. **32**:334, 1941.
46. Councilman, W. T.: J. Exper. Med. **3**:393, 1898.
47. Brody, H., and Smith, L. W.: Am. J. Path. **12**:373, 1936.
48. Bell, E. T.; Clawson, B. J., and Hartzell, T. B.: Am. J. Path. **1**:247, 1925.
49. Stoddard, J. L., and Woods, A. C.: J. M. Research **34**:343, 1916.
50. Lukens, F. W. D., and Longcope, W. T.: Bull. Johns Hopkins Hosp. **53**:511, 1931.

soluble toxin found in broth cultures of streptococci is antigenic and therefore probably a protein. This was shown for the streptococcus of erysipelas by Birkhaug[51] and for the streptococcus of scarlet fever by Dick and Dick.[52] If the antigen (streptococcus toxin) is a colloid, it appears possible that it might, when injected into the circulating blood in sufficient amount and concentrated in the glomeruli, directly injure this part of the renal units.

We injected streptococcus toxin into 6 dogs. Four of these dogs were given from two to six doses of the toxin of the streptococcus of erysipelas, the doses being 0.2 and 0.25 cc. per hundred cubic centimeters of blood. The other 2 dogs received 6 doses each of the toxin of the streptococcus of scarlet fever, the doses being 2,000 and 5,000 cutaneous test doses, respectively, per hundred cubic centimeters of blood. The animals were killed from one to four days after the administration of the first dose. Of these 6 dogs, 5 had albumin in the urine, ranging from a trace to 2 plus.

The glomeruli of 2 dogs contained marked amounts of fat, and in the same animals there was fat in the upper segments of the proximal convoluted tubules. In all these dogs there was engorgement of the glomerular capillaries, but this was prominent in only 3. Proliferation of the endothelium of the glomerular capillaries was slight or questionable. In 4 dogs there was some disintegration of nuclei in the glomeruli. Cloudy swelling of the tubular epithelium was present in all dogs of this group but was marked in only 2. In the latter 2 dogs there was a moderate amount of necrosis of the tubular epithelium. In 2 dogs the intertubular capillaries were congested. In the kidneys of 2 dogs, one injured with the toxin of the streptococcus of erysipelas, and the other with that of the streptococcus of scarlet fever, foci of interstitial lymphocytic and plasma cell infiltration were found.

In all these dogs the changes described were moderate in degree but those in the animals given erysipelas streptococcus toxin were definitely more prominent. Five thousand cutaneous test doses of the scarlet fever streptococcus toxin produced more injury in the kidneys than did 2,000 such doses. The small number of dogs and the limited variation in the dosage employed do not warrant drawing definite conclusions concerning the effects of streptococcus toxin on the kidneys. Our results are presented as indicating the possibilities and the limitations of streptococcus toxin as a nephrotoxic agent.

COMMENT

Theoretically, a colloidal poison introduced into the circulating blood in optimal doses might be expected to become sufficiently concentrated, through loss of water by filtration, to injure the glomeruli. The chief obstacles to the successful production of experimental glomerulonephritis arise from the lack of a colloidal poison ideal for this purpose and from the difficulties inherent in identifying and accurately evaluating the changes produced in the glomeruli.

Snake venoms are complex substances. They consist, at least in the crude form, of proteins (both albumins and globulins), lipoproteins, proteoses and peptones, mucin and mucin-like substances, detritus of

51. Birkhaug, K. E.: Bull. Johns Hopkins Hosp. **36**:248, 1925; **37**:85, 1926.
52. Dick, G. F., and Dick, G. H.: J.A.M.A. **82**:265 and 544, 1924.

cells and various salts.[53] Micheel and Kraft [54] expressed the belief that native venoms consist largely of proteins and related substances which do not pass through cellophane. But they also considered the possibility that the poisonous part of venom might be a prosthetic group that requires a colloidal carrier. Peck and Marx [55] found that the hemorrhagin and the hemolysin are in the protein fraction of venom and that as regards cellophane they are practically nondialyzable.

Snake venom, therefore, appears to meet one of the requirements of a colloidal poison that might be used to produce experimental glomerulonephritis. However, as shown in our experiments, the constant damage of the tubular epithelium that equaled or exceeded the visible injury of the glomeruli, the intravascular hemolysis indicated by the frequent presence of hemoglobinuria, the definite and sometimes striking loss of body weight, the changes in the liver weight–body weight ratios in our dogs and the narrow range between the minimum lethal dose and the nephrotoxic dose as pointed out by Eisenbrey,[15] all indicate that snake venom is not suitable for the production of experimental glomerulonephritis.

Diphtheria toxin was held by Pearce [56] to be the best of the agents thus far used to produce glomerular damage. But it lacks much of being an ideal agent. It damages the tubules as much as, or more than, it injures the glomeruli. A dose of this toxin that will visibly injure the kidneys will also affect other organs, as shown by the increase in liver weight–body weight ratios mentioned in a preceding paragraph. The action of diphtheria toxin is not as constant, its effects cannot be measured with the same degree of accuracy, the results of its injection cannot be predicted with as much certainty, and the changes produced are not as distinct and definite as those produced by crystalloidal, inorganic agents used on other dogs in this series of experiments.

A similar objection applies to staphylococcus toxin. It is such a powerful epithelial poison that it is difficult to select a dose that will injure the glomeruli without at the same time producing extensive necrosis of the tubular epithelium. The molecule of this toxin, although undoubtedly protein since it induces the formation of antibodies, is apparently relatively small and passes through an even slightly damaged glomerular filter. This is the probable explanation of the extensive

53. Do Amoral, A.: Snake Poisoning, in Nelson's Loose Leaf Medicine, New York, Thos. Nelson & Sons, 1932, vol. 2, chap. 5, p. 683.

54. Micheel, F. and Kraft, K., cited by Medes, G., in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1939, vol. 8, p. 196.

55. Peck, S. M., and Marx, W. J.: J. Mt. Sinai Hosp. **6**:171, 1941.

56. Pearce, R. M.: Arch. Int. Med. **5**:133, 1910.

necrosis of the tubular epithelium with which it comes in contact in a concentrated form because of absorption of water.

In view of the frequency with which glomerulonephritis seems to follow streptococcic infections, efforts to injure the glomeruli with streptococcus toxin have been particularly disappointing. The renal changes induced were relatively minor.

Numerous chemical agents in adequate doses will injure the glomeruli. Even crystalloidal poisons, such as uranyl nitrate, if present in the plasma in sufficient concentration (3 mg. or more per hundred cubic centimeters of blood) will cause visible damage to the glomeruli. Cantharidin has been frequently used as a glomerular poison, but this substance also injures the tubules.[57] If the glomeruli are to be injured by a poison concentrated in the plasma through loss of water by filtration, the poison must be colloidal in nature. The colloidal poison ideal for this purpose must have at least four characteristics or qualities:

1. The molecule of the ideal colloidal nephrotoxic agent must be of a size to prevent its passage through the glomerular filter. Little or nothing is known concerning the size of the smallest molecule that is retained by the glomerular filter. Phosphatase, with a molecular weight between 6,000 and 10,000,[58] and inulin, with a molecular weight of 5,000 [59] but with a diffusion coefficient equivalent to a molecular weight of 15,000,[60] pass readily through the walls of the glomeruli. We have no adequate knowledge concerning the size of the molecules of the colloidal poisons used in our experiments. We believe that all of them are colloids and protein in nature because each of them will induce the formation of antibodies when injected into an animal. Gralen and Svedberg [61] found the molecular weight of purified crotoxin, the neurotoxic venom of Crotalus terrificus, to be about 30,000. Patermann and Pappenheimer [62] determined the molecular weight of diphtheria toxin to be 74,000. The molecular weight of Lancefield's [63] toxic "M substance" of streptococcus was found to be 41,000.[64] If the colloidal poisons used by us had molecular weights no greater than those mentioned, their molecules were

57. MacNider, W. DeB.: Physiol. Rev. **4**:595, 1924. Folin, O.; Karsner, W. T., and Denis, W.: J. Exper. Med. **16**:789, 1912. Eisenbrey.[15] Lyon.[20]

58. Albers, H., and Albers, E.: Ztschr. f. physiol. Chem. **232**:165 and 189, 1935.

59. Westfall, B. B., and Landis, W. M.: J. Biol. Chem. **116**:727, 1936.

60. Bunim, J. J.; Smith, W. W., and Smith, H. W.: J. Biol. Chem. **118**:667, 1937.

61. Gralen, N., and Svedberg, T.: Biochem. J. **32**:1375, 1938.

62. Patermann, M. L., and Pappenheimer, A. M., Jr.: J. Physiol. Chem. **45**:1, 1941.

63. Lancefield, R. C.: J. Exper. Med. **47**:91, 843 and 859, 1928.

64. Pappenheimer, A. M., Jr.; Williams, J. W., and Zittle, C. A.: J. Immunol. **43**:61, 1942. Zittle, C. A., and Mudd, S.: Ann. New York Acad. Sc. **43**:47, 1942.

small in comparison with those of other proteins, such as serum albumin. Even very large molecules of foreign proteins, such as egg albumin, when introduced into the circulation are, it is known, excreted by the glomeruli. But Babcock [65] has shown that such foreign proteins first injure the glomeruli and thus increase the permeability of these structures before being excreted.

2. The toxicity of the ideal colloidal nephrotoxic poison must be such that an amount introduced into the circulating blood which will not damage other organs will be sufficiently concentrated in the glomeruli through loss of water by filtration to produce local injury. That is, there must be a wide range between its minimum nephrotoxic dose and its minimum lethal dose. Ricin, a vegetable protein with a large molecule, is far too toxic to be an ideal colloidal poison for this purpose.[66] In our experience rattlesnake and moccasin venoms possess the same objectionable quality.

3. The ideal colloidal nephrotoxic poison must not produce undesired side effects. Some of these effects are inherent in the nature of the particular poison. For example, the molecule of staphylococcus toxin is relatively small. The small doses of this poison used by us appear to have increased the permeability of the glomeruli adequately to permit passage of the poison through the glomerular filter but not enough to produce appreciable amounts of albuminuria. Only 5 of our 12 dogs given this toxin showed albumin in the urine and these only from a trace to 2 plus. And yet the tubular epithelium in these dogs was sufficiently necrotic to indicate that the poison had passed through the glomerular walls with the filtrate. But even the largest molecule of a colloidal poison might ultimately injure the glomeruli sufficiently to permit it to escape into the filtrate and thus damage the tubular epithelium. Furthermore, a colloidal poison that had been sufficiently concentrated in the glomeruli by filtration to injure the endothelium would pass, in its newly concentrated form, with the blood into the peritubular capillaries, where it might be expected to produce further damage of these structures. Faber [2] has called attention to this possibility and has pointed out difficulties in the actual demonstration of the lesion.

4. The ideal colloidal nephrotoxic agent must produce lesions in the glomeruli that can be readily demonstrated and evaluated. The difficulty here lies in the marked differences of the structural patterns of the two parts of the renal units. The tubule is a relatively simple structure, consisting of cells arranged about a lumen. The glomerulus, on the other hand, is composed of about 50 long capillaries with their two ends

65. Babcock, C.: Anat. Rec. **71**:233, 1938.

66. Ford, W. W.: Centralbl. f. Bakt. (Abt. 2) **58**:129, 1913.

anchored at the hilus of the glomerulus and the remainder forming a mass of loops lying in the capsular space. These capillaries thus form a twisted skein in which it is next to impossible to differentiate separate loops. The changes which occur in them produce a mass effect rather than separate and distinct lesions that can be localized in individual cells and blood vessels. Thus it is far more difficult to visualize and describe definite pathologic changes in the glomeruli than in the tubules. It is therefore extremely difficult to standardize either the dose or the effects of a glomerular poison.

Certain shortcomings of an ideal colloidal nephrotoxic poison are inherent in its very nature. Its tendency to escape through injured glomeruli in the filtrate and to injure the tubular epithelium and the likelihood of its injuring the endothelium of the peritubular capillaries after being concentrated in the glomeruli have been mentioned. But a colloidal poison in the circulating blood differs from an inorganic, crystalloidal poison in an important respect. The inorganic nephrotoxic agent may combine with the proteins of the plasma, but it apparently leaves the blood stream only through certain excretory organs, of which the kidney is the most important. A colloidal poison, on the other hand, is rapidly removed from the blood by the reticuloendothelial system. This rapid change in the concentration of a colloidal poison in the blood stream renders the effects of such poisons uncertain and unpredictable. We attempted to obviate this difficulty by blockading the reticuloendothelial system of some dogs with india ink, but the results were not convincing.

SUMMARY

The experiments reported in this paper were based on the hypothesis that a dose of a colloidal poison could be found that when introduced into the circulating blood in amounts too small to injure other organs might be concentrated in the glomeruli through loss of water by filtration so as to injure these structures.

An ideal nephrotoxic colloidal poison should have the following characteristics: (*a*) its molecule should be large enough that it will not pass through the normal glomerular filter; (*b*) there must be a wide range between its minimum nephrotoxic dose and its minimum lethal dose; (*c*) it must not produce undesired side effects; (*d*) it must produce lesions in the glomeruli that can be readily demonstrated and evaluated.

In our experiments we used rattlesnake and moccasin venoms, the toxin of Corynebacterium diphtheriae and streptococcus and staphylococcus toxins. None of these proved to be an ideal colloidal nephrotoxic agent.

The venoms used in these experiments were furnished by Sharp & Dohme, Inc., and the bacterial toxins, by Parke, Davis & Company.

PITUITARY LESIONS ACCOMPANYING OBESITY

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THE association of anatomic lesions of the pituitary gland and obesity has been noted in a number of conditions, such as Fröhlich's syndrome, Cushing's disease and tumors of the pituitary gland. Even with the rare types of obesity a variety of changes of this gland have been found. In Dercum's disease adenocarcinoma,[1] glioma,[2] adenoma or adenomatous hyperplasia,[3] and fibrosis [4] have all been described. Winkelman and Eckel [5] found various pathologic changes in 8 of 11 pituitary glands which they examined. In Morgagni's syndrome adenomatous hyperplasia [6] and increase of the basophilic cells [7] have been reported. In Günther's syndrome [7] and in the Laurence-Moon-Biedl [8] syndrome increase of the basophilic cells has been found. Simmonds' disease due to postpartum necrosis of the pituitary gland has been observed associated with considerable gain in weight during the early stages,[9] but the same gain in weight is also found in an apparently normal postpartum course.[9] Trauma to the pituitary gland in man has been followed by obesity, according to Marañon (quoted by Echauz [10]). The evidence for this conclusion is purely circumstantial and dubious at best. The occurrence of obesity after experimental injury has been both advocated [11] and disputed.[12]

Of greater practical importance, perhaps, is the vast number of cases of obesity which do not fall into any of the aforenamed groups but are classified simply as cases of "constitutional" or "endocrine

From the Laboratory of Pathology, City Hospital, Welfare Island.

1. Dercum, F. X., and McCarthy, D. J.: Am. J. M. Sc. **124**:994, 1902.
2. Burr, C. W.: J. Nerv. & Ment. Dis. **27**:519, 1900.
3. Price, G. E.: J. Nerv. & Ment. Dis. **36**:158, 1909. Foot, N. C.; Good, R. W., and Menard, M. C.: Am. J. Path. **2**:251, 1926.
4. Guillain, G., and Alquier, L.: Arch. de méd. expér. et d'anat. path. **18**: 680, 1906.
5. Winkelman, N. W., and Eckel, K. L.: J. A. M. A. **85**:1935, 1925.
6. Henschen, F.: Hygiea **98**:65, 1936.
7. Ritter, W.: Frankfurt. Ztschr. f. Path. **52**:149, 1938.
8. Anderson, N. L.: J. Clin. Endocrinol. **1**:905, 1941.
9. Sheehan, H. L.: Quart. J. Med. **8**:277, 1939.
10. Echauz, F.: Arch. de med., cir. y especialid. **31**:606, 1929.
11. Biancardi, S.: Fisiol. e med. **2**:267, 1931.
12. Hetherington, A. W.: Endocrinology **26**:264, 1940.

obesity." Here, even in cases labeled "hypopituitary obesity" the diagnosis is based on the distribution of fat deposits, the characteristic metabolic pattern [13] and other clinical manifestations.[14] "Hypopituitary obesity" accompanied by retention of sodium and water has been ascribed by one writer [14a] to space-consuming intrasellar lesions—the "subclinical adenomata" of Costello.[15] All these concepts, however, are based almost exclusively on clinical evidence. Morphologic studies are relatively scanty, since obesity is rarely a fatal disease and is seldom seen at the autopsy table, particularly in persons who are in the early decades of life. Cases which have had a thorough metabolic study are even rarer.

The pathologic changes noted in the pituitary gland in "constitutional obesity" have been mainly changes in the proportions between the cell types, hyperplasias, benign tumors and stromal increase. Increase in the basophilic cells appears to be the most frequently observed feature; the range of incidence, however, is wide. Kraus [16] noted it in 81.8 per cent of his cases, Zeynek [17] in 81.3 per cent (26 of 32 cases), Bini [18] in 38.9 per cent (8 of 21 cases), Guizzetti [19] in 20 per cent (3 of 15), Kiyono [20] in 17 per cent (1 of 6) and Ritter [7] in a single case. Kraus expressed the opinion that this change was nonspecific since the identical finding is frequent in hypertension or renal disease. The eosinophilic cells were noted as increased in 1 case by Kiyono and decreased in 14 cases by Zeynek. The chromophobe cells have likewise been reported either as increased (Zeynek; Kiyono) or decreased (Zeynek). Adenomatous hyperplasia was found by Zeynek in 5 cases, by Guizzetti in 1 and by Kiyono in 1. Adenomas have been seen by all observers. Zeynek reported them in 6 cases, Guizzetti in 3 and Bini in 4. The adenomas have been of various types: chromophobe (Guizzetti; Bini), eosinophilic (Guizzetti), basophilic (Zeynek; Bini), "indifferent" (Bini) and choristoma (Bini).

Stromal increase has been noted by several observers. Zeynek found a "prominent increase" in 3 cases. Kup [21] noted "circumscribed fibrosis of the anterior lobe" of the pituitary gland of a 38 year old

13. Goldzieher, M. A.; Reimer, N. A., and Goldzieher, J. W.: Am. J. Digest. Dis. **12**:387, 1945.

14. (*a*) Goldzieher, M. A.: The Endocrine Glands, New York, D. Appleton-Century Company, Inc., 1939, p. 310. (*b*) Gottesman, A.: Med. Klin. **28**:15, 1932. (*c*) Lhermitte, V.; de Massary, V., and Trelles, J. O.: Rev. neurol. **1**:375, 1934.

15. Costello, R. T.: Am. J. Path. **12**:205, 1936.

16. Kraus, J. E.: J. Clin. Endocrinol. **5**:42, 1945.

17. Zeynek, E.: Frankfurt. Ztschr. f. Path. **44**:387, 1933.

18. Bini, G.: Endocrinol. e pat. costit. **11**:168, 1936.

19. Guizzetti, F.: Arch. per le sc. med. **57**:325, 1933; **58**:1, 1934.

20. Kiyono, H.: Virchows Arch. f. path. Anat. **259**:388, 1926.

21. Kup, J.: Endokrinologie **6**:102, 1930.

woman. Kiyono noted a "sclerotic focus" in 1 case. Guizzetti described "senile fibrosis" in 7 and Bini "considerable fibrosis" in 5.

A number of other changes have been reported. In Kup's series of 31 pituitary glands 4 weighed over 1.0 Gm., 4 presented colloid cysts and 1 showed a chronic nonspecific granuloma. Marked increase in colloid was noted in isolated cases of extreme obesity by Kraus.[22] Evidence of gross compression was seen by Guizzetti in 3 cases; Bini found simple atrophy in 1, and Goldzieher[14a] observed degenerative changes and interstitial fibrosis with or without round cell infiltration to be frequent. Kiyono could find no relationship between obesity and metastatic lesions of the hypophysis in cases of carcinoma of the breast.

MATERIALS AND METHODS

The material consisted of the pituitary glands of 10 patients who came to autopsy at City Hospital, Welfare Island, New York, during the past five years. In each case the obesity was extreme; the distribution of fat was not described in sufficient detail for one to judge accurately whether it conformed to the various clinical types of "endocrine obesity." A few clinical and laboratory data were available in 2 instances only.

As control material 9 pituitary glands were used from persons who had no physical or historic evidence of past or present obesity, who had disease processes as similar as possible to those found among the obese, and who corresponded closely in age and sex.

The tissues were fixed in 10 per cent solution of formaldehyde, embedded in paraffin and stained with hematoxylin and eosin, the Heidenhain azocarmine modification of the Mallory connective tissue stain, a modified Mallory acid fuchsin stain, chromium-hematoxylin of Kraus[23] for acidophilic granules and cresofuchsin of Erdheim and Stumme[24] for basophilic granules. The steps of the modified Mallory acid fuchsin method are appended, as are those of the Kraus and Erdheim-Stumme methods, since descriptions of these methods, as far as we know, are not available in the American literature.

RESULTS

The frequency of changes in the ratio of the various cell types was highly suggestive. In all 10 of the obese persons there was an increase of the basophilic cells of the pituitary gland. This change was marked in 6 and moderate in 4. The eosinophils were decreased in 2. There was pronounced increase of the chromophobes in 5.

Small adenomas of the gland were found in 4 obese persons. In 2 they were chromophobe (patients 1 and 10 in table 1); in 2, basophilic (patients 5 and 6).

Senile fibrosis, shown by the prominence of the "core," was present in 3 obese persons (7, 8 and 10). On the other hand, a very peculiar,

22. Kraus, E. J., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, p. 810.

23. Kraus, E. J.: Frankfurt. Ztschr. f. Path. **10**:161, 1912.

24. Erdheim, J., and Stumme, F.: Virchows Arch. f. path. Anat. **46**:1, 1909.

relatively circumscribed scarring, quite different from the senile changes, was found in 9 patients, being absent in only 1 (patient 2).

A marked increase in colloid of the pituitary gland was found in 3 obese persons; a moderate increase, in 1. Pronounced vacuolation of the basophilic cells was present in 6 of the obese persons; in 3 others this change was present to a slight extent.

The pertinent clinical and anatomic data on the obese persons are summarized in table 1 and the reports of 2 cases of obesity are given in detail.

CASE 1.—A 28 year old white woman entered the hospital because of multiple thrombophlebitis. The past history revealed a sudden gain in weight at the age

TABLE 1.—*Cases of Obesity*

Patient	Sex	Age	Pathologic Diagnosis	Body Proportions	Other Data
1	F	28	Thrombophlebitis migrans; rheumatic heart disease	Tremendously obese	Asthma; headaches; hyperorexia; hypertension
2	F	47	Cardiac hypertrophy; passive congestion; adenoma of adrenal cortex; hernia; bronchopneumonia	Extremely obese; breasts huge; abdominal fat 5 inches (12.5 cm.) thick	Asthma; cholecystitis; sporadic amenorrhea
3	F	46	Carcinoma of breast with metastases; slightly nodular adrenal gland	Short (5 ft. 3 in. [160 cm.]), very obese	Hirsuties of lip and chin
4	F	45	Thrombosis of internal carotid artery; cholelithiasis; fatty heart and liver	Obese	Chronic alcoholism some loss of weight
5	F	50	Choledocholithiasis; acute myocarditis; bronchopneumonia	Extremely obese, including abdomen, neck and legs	Menopause at 42
6	F	69	Adenoma of bronchus; bronchiectasis; cholelithiasis	Extremely obese; breasts and abdomen pendulous	
7	F	60	Atherosclerosis; cerebral hemorrhage	Extremely obese trunk; slender extremities	Hypertension
8	F	70	Bronchopneumonia	Extremely obese	Very mild diabetes
9	M	79?	Carcinoma of prostate; portal cirrhosis	Markedly obese	Very mild diabetes; rheumatoid arthritis
10	F	80?	Cholangiohepatitis; bronchopneumonia	Very obese, short	Blood sugar, 75; cholesterol, 182

of 17, which had progressed to extreme obesity. Asthma developed at 20 years. There were frequent headaches; the menses were irregular and scanty; the appetite was admittedly excessive. The blood pressure ranged from 180 systolic and 120 diastolic to 250 systolic and 120 diastolic. The fasting blood sugar was 114 mg. per hundred cubic centimeters. The blood cholesterol was not elevated. Death was due to multiple migrating thrombophlebitis causing infarcts of the lungs and the spleen. Autopsy revealed, in addition, severe cardiac hypertrophy and evidence of rheumatic heart disease. A complete report of the case has been published elsewhere.[25]

Pituitary Gland.—The pituitary gland did not appear enlarged. The microscopic observations were as follows: The capsule was thin. The acini were large and cellular. There was an irregular, marked increase of the stroma

25. Swirsky, M. Y., and Cassano, C. C.: J. Lab. & Clin. Med. **28**:1812, 1943.

throughout with occasional frank scars, which were easily distinguished from the physiologic "core." Colloid was minimal. The eosinophils were numerous and of average size; they showed no changes of nucleus or cytoplasm. The basophils were definitely increased in number throughout the gland except perhaps at the posterior aspect, where eosinophils crowded them out. In the scarred areas basophils were present almost exclusively, and a suggestion of pseudopapillary formation was noted. A small proportion of the basophils showed vacuoles within the cytoplasm, but this was not a striking feature. The chromophobe cells seemed somewhat increased in number. There was a circumscribed adenoma of chromophobe cells just anterior to the intermediate zone; it measured 2.5 by 4.0 mm. in cross section. The surrounding tissue showed evidence of compression flattening, while the nodule itself manifested regressive changes.

Case 2.—A 47 year old white woman entered the hospital because of cardiac failure. The past history revealed the following facts: Her weight at 16 years was 125 pounds (56.5 Kg.); at 20 years it was 150 pounds (68 Kg.); a marked gain began shortly after the birth of her first child. The weight at the time of admission was approximately 350 pounds (158.5 Kg.). There had been amenorrhea alternating with occasional menorrhagia for a long time; asthma was present; cholelithiasis had necessitated removal of the gallbladder. The blood pressure ranged about 260 systolic and 140 diastolic. The fasting blood sugar was 110 mg., the uric acid 3.3 mg. and the cholesterol 125 mg. per hundred cubic centimeters. The roentgenographic appearance of the sella turcica was said to be normal. Death was due to intractable cardiac failure and bronchopneumonia. Autopsy revealed, in addition to general passive congestion, marked hypertrophy of the right and left sides of the heart, a small adenoma of the cortex of the adrenal gland and an incarcerated hernia.

Pituitary Gland.—The capsule was thin. The gland appeared rather cellular, especially as the cells generally were rather small and the acini not always distinct. The stroma was not at all prominent save for a single extension of the core far into the anterior lobe. Colloid was minimal. The eosinophils were present in fair numbers, and no changes of nucleus or cytoplasm were seen. The basophils were markedly increased in patchy fashion, forming definite nests of cells. Vacuoles were seldom large and were found only in a small proportion of the cells. The chromophobes were definitely increased in number, and this change was most prominent in those areas showing a predominance of basophils.

The observations in the 2 cases just described serve to illustrate the fact that the changes observed are by no means predictable. The history of case 1 is not entirely typical of "hypopituitary obesity," whereas that of case 2 is far more suggestive. The results of the laboratory studies which were made do not conform to the metabolic pattern of "hypopituitary obesity" in either case. Yet rather pronounced changes were seen in case 1, whereas increases of the basophils and chromophobes were the only essential findings in case 2.

Controls.—In the 9 control pituitary glands a slight increase of the basophils was found in a single gland; no increase was seen in the remainder. No noteworthy changes of the eosinophils or of the chromophobes were found in any of the controls. No adenomas were found. Increased colloid was noted in 5; this was marked in 3 and moderate in 2. Vacuolation of the basophilic cells was marked in 5 glands and slight in 1. The pertinent clinical and anatomic data on the controls are summarized in table 2.

COMMENT

The most interesting feature found in this series was the stromal change which appeared to be so characteristic. The connective tissue of the hypophyses which I have examined falls into three distinct categories. There is the physiologic stroma—the so-called "core"—which is seen in the region between the anterior and the intermediate lobe and which from there sends extensions forward. The connective tissue is of the characteristic hyaline variety with typical cigar-shaped nuclei and thick, wavy bundles of collagenous fibers (fig. 1). In addition, blood vessels and lymphatics accompany these strands and thereby make their identification easier. It is my impression that the connective tissue of the core increases, or at least becomes more prominent, with age; the essential characteristics, however, do not change.

TABLE 2.—*Controls*

Patient	Sex	Age	Pathologic Diagnosis	Body Proportions	Other Data
1	F	49	Hepatic cirrhosis; ruptured esophageal varices	Average	
2	F	49	Pyelonephritis; multiple sclerosis	Average	Blood chemically normal
3	F	60	Cirrhosis; esophageal varices	Somewhat emaciated	Chronic alcoholism
4	F	62	Pulmonary embolism; calcified colloid goiter	Average	
5	F	60+	Miliary tuberculosis; cholelithiasis	Emaciated	
6	F	65	Radical mastectomy; coronary arteriosclerosis	Emaciated	Hypertension; old hemiplegia
7	F	68	Cardiac thrombus; emboli	Average	
8	M	74	Paget's disease; cholelithiasis	Average	
9	F	78	Bronchopneumonia	Emaciated	Mild diabetes

In the process of aging the interacinous stroma also becomes more prominent. This tissue shares many of the characteristics of the core, having a tendency to run in strands and showing numerous, occasionally somewhat vesicular nuclei. The fibers are rather finer than those of the core, although there is still a tendency to group into bundles (fig. 2). In all the controls in which an increase of stroma was noted, the change falls into one of the aforementioned groups.

On the other hand, 9 of the glands of the obese patients showed a strikingly different change. Here were observed localized, prominent increases of the connective tissue suggestive of small scars (fig. 3). The stroma in these areas was quite different from that described in the foregoing paragraph. The collagenous tissue contained but few nuclei, its fibrils were delicate and nonhyaline, and the adjacent parenchyma showed changes as well (fig. 4). Here the acini tended to be atrophic except where they were replaced by areas of almost adenom-

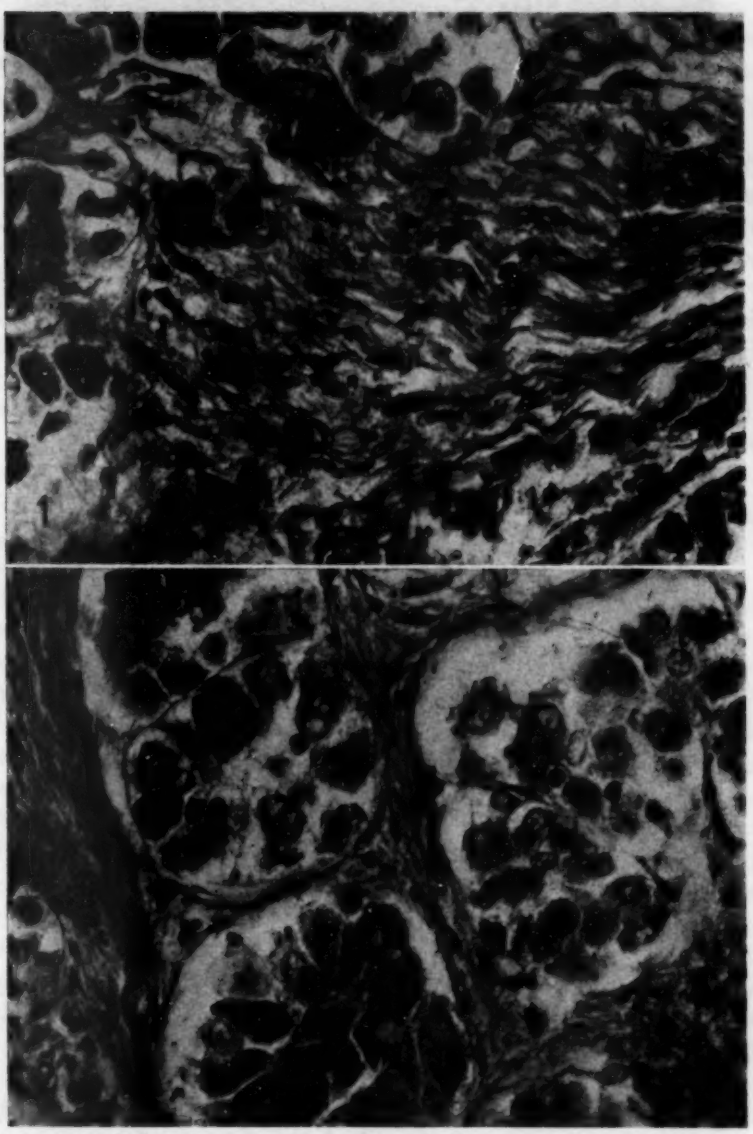

Fig. 1.—Section of the physiologic "core" of the pituitary gland, showing the wavy bundles of hyaline connective tissue, the numerous typical connective tissue cell nuclei and a blood vessel. Modified Mallory acid fuchsin connective tissue stain. × 450.

Fig. 2.—Senile fibrosis. Note the amply nucleated bundles of slightly wavy collagenous fibers and the large acini containing a variety of healthy-looking cells. Modified Mallory acid fuchsin connective tissue stain. × 450.

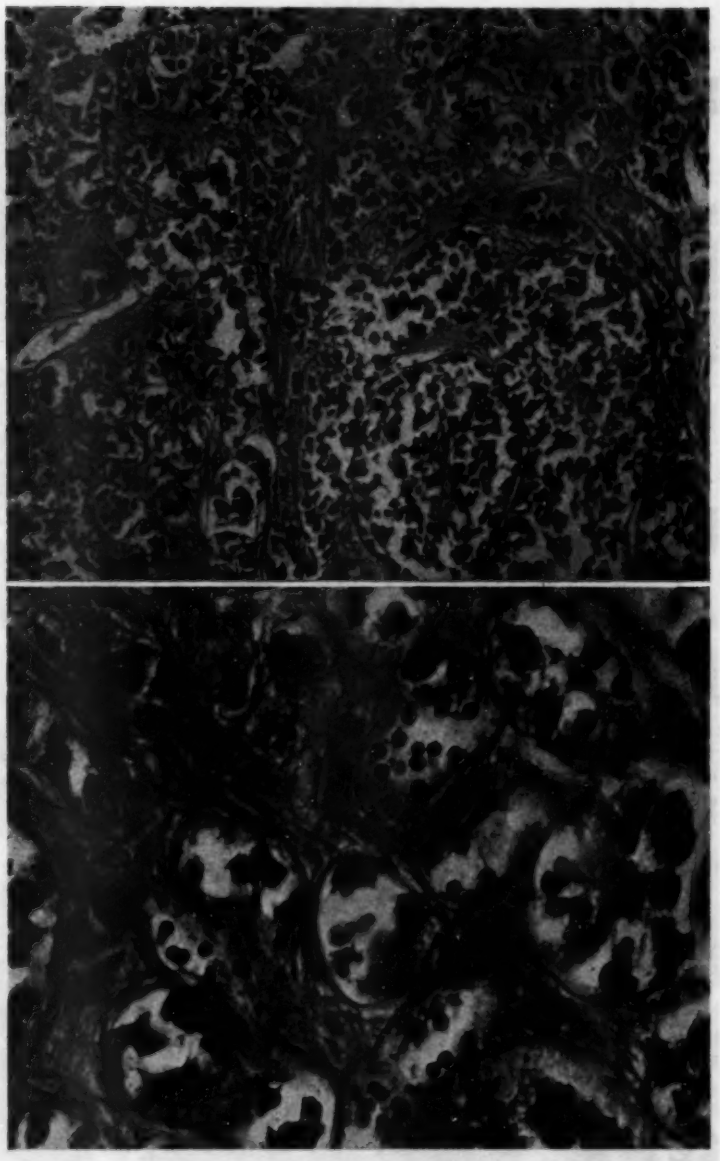

Fig. 3.—Circumscribed area of diffuse interstitial fibrosis with atrophic acini (at left) giving way to markedly hyperplastic areas (at lower right). Modified Mallory acid fuchsin connective tissue stain. × 140.

Fig. 4.—High power view of a part of the section shown in figure 3. Note the finely fibrillar character of the scar tissue, the absence of nuclei or of hyaline appearance. The acini are atrophic and consist exclusively of basophilic cells. Moderate congestion is evident. Modified Mallory acid fuchsin connective tissue stain. × 450.

atous hyperplasia. The cells in these scarred areas were decreased in size and nearly always basophilic in character. In some hyperplastic areas the proliferation assumed almost pseudopapillary proportions.

The presence of focal scarring has been described by a number of the authors cited; most of them dismiss the change as senile atrophy. It is possible that no distinction was made between true senile fibrosis and the morphologically quite different areas of scarring which I observed only in the pituitary glands of obese persons and never in the controls falling into the "senile" age group. Moreover, advanced parenchymal changes in the vicinity of these peculiar scars suggest that there may have been qualitative as well as quantitative change in the local secretory activity.

The present material shows the predominance of basophil cells noted by others. It does not, however, suggest that there is any correlation between obesity and the accumulation of colloid, as this change was noted in equal proportions of the glands of obese persons and controls. The vacuolation of the basophilic cells also does not differ markedly in the two groups.

An incidence of adenoma in 40 per cent in the group of obese persons as against none in the controls is entirely within the range of statistical variation in such small samples and hence is not significant. However, in adding together this material and that of Bini, Guizzetti and Zeynek I find an incidence of 23 per cent (18 of 78 cases). This is almost identical with the figure of Costello—22.5 per cent—arrived at from a study of a thousand routinely received pituitary glands. Although it would be valuable to have a statistical breakdown of Costello's data as regards the presence or the absence of obesity, the similarity of his figure to the incidence I have just calculated apparently does not support the hypothetic role of the subclinical adenoma in the causation of obesity.

The interpretation of these findings and their relation to obesity and the metabolic status generally must be approached with great caution. There is no evidence to prove that the scarring as an indication of pituitary damage is causally related to obesity. On the other hand, one should not fall prey to the post hoc fallacy and assume that these changes are the consequence of the metabolic disturbances. With the available data, the association of these two facts can merely be noted; no conclusions may be drawn.

It is obvious that the material studied under the generic term "constitutional" or "endocrine obesity" is a heterogeneous group, and consequently a uniform and characteristic appearance of the pituitary glands is hardly to be expected. Moreover, it must be conceded that functional alterations of the pituitary gland not detectable by present day histologic technics are entirely possible. The final answer to this problem will rest in the accumulation of cases in which the results of

careful histologic study of the pituitary gland can be correlated with the clinical history, the distribution of fat and the metabolic pattern.

SUMMARY

In a series of 10 cases of "constitutional obesity" the pituitary changes consisted of a peculiar scarring of the stroma and a predominance of the basophils with some increase in the chromophobes. No such changes were seen in 9 selected controls. Although this feature of the pituitary glands of obese persons was striking, the question of its causal relation to the development of obesity must remain in abeyance. The term "constitutional" or "endocrine obesity" appears to comprise a heterogeneous group of cases.

APPENDIX

1. *Modified Mallory Acid Fuchsin Connective Tissue Stain:*
   1. Deparaffinize and stain with Delafield's hematoxylin ten to fifteen minutes, followed by acid alcohol and then by weak ammonia water.
   2. Stain with 0.1 per cent acid fuchsin for thirty seconds to one minute.
   3. Stain in Mallory's aniline blue–orange G–phosphomolybdic acid ten to twenty minutes.
   4. Dehydrate and clear in ascending alcohols and xylene then mount in balsam.
2. *Kraus's Chromium-Hematoxylin Stain for Acidophilic Granules:*
   1. Deparaffinize and mordant overnight in 5 per cent aqueous potassium dichromate.
   2. Stain in Kultschitzky hematoxylin for twenty-four hours. (This is prepared by dissolving 2.0 Gm. of hematoxylin in sufficient dehydrated alcohol, then making the solution up to a volume of 100 cc. with 2 per cent acetic acid. Ripening is necessary.)
   3. Wash in water briefly.
   4. Differentiate for fifteen minutes to two hours in a 1:1 dilution of Weigert's differentiator (borax 2.0 Gm., potassium ferricyanide 2.5 Gm., water 100 cc.).
   5. Counterstain with picrofuchsin if desired.
3. *Erdheim-Stumme Cresofuchsin Stain for Basophilic Granules:*
   1. Deparaffinize. Overstain heavily with Orth's lithium carmine [26] and wash in water.
   2. Stain for twenty-four hours in a closed container of cresofuchsin solution (cresofuchsin 0.3 Gm., concentrated hydrochloric acid 2 cc., 70 per cent ethyl alcohol 100 cc.).
   3. Wash in 70 per cent alcohol, clear in carbolxylene and xylene and mount in balsam.

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26. If counterstaining is not desired, Orth's carmine may be eliminated.

Case Reports

ADENOACANTHOMA OF THE STOMACH

GEORGE STRASSMANN, M.D., WALTHAM, MASS.

HETEROLOGOUS adenoacanthoma (Pasternack [1]), or adenocancroid (Herxheimer [2]), a mixed glandular and squamous cell carcinoma of the stomach, is rare. There is variance of opinion in explaining the occurrence of tumors of this type, which arise in areas where normally no squamous cell epithelium is present. In a recent review Wood [3] collected 10 cases from the literature and added 2 of his own. The fact that in only 2 of the 12 cases the tumor was situated in an extrapyloric region at some distance from the cardia (Martin and Pollosson [4]; Scheffler and Falk [5]) indicates that adenoacanthoma has a predilection for the pyloric region. In 1 instance adenoacanthoma extended from the pylorus to the cardia (Takagi [6]). The case now reported is the second instance of adenoacanthoma extending beyond the pyloric area to the cardia.

REPORT OF A CASE

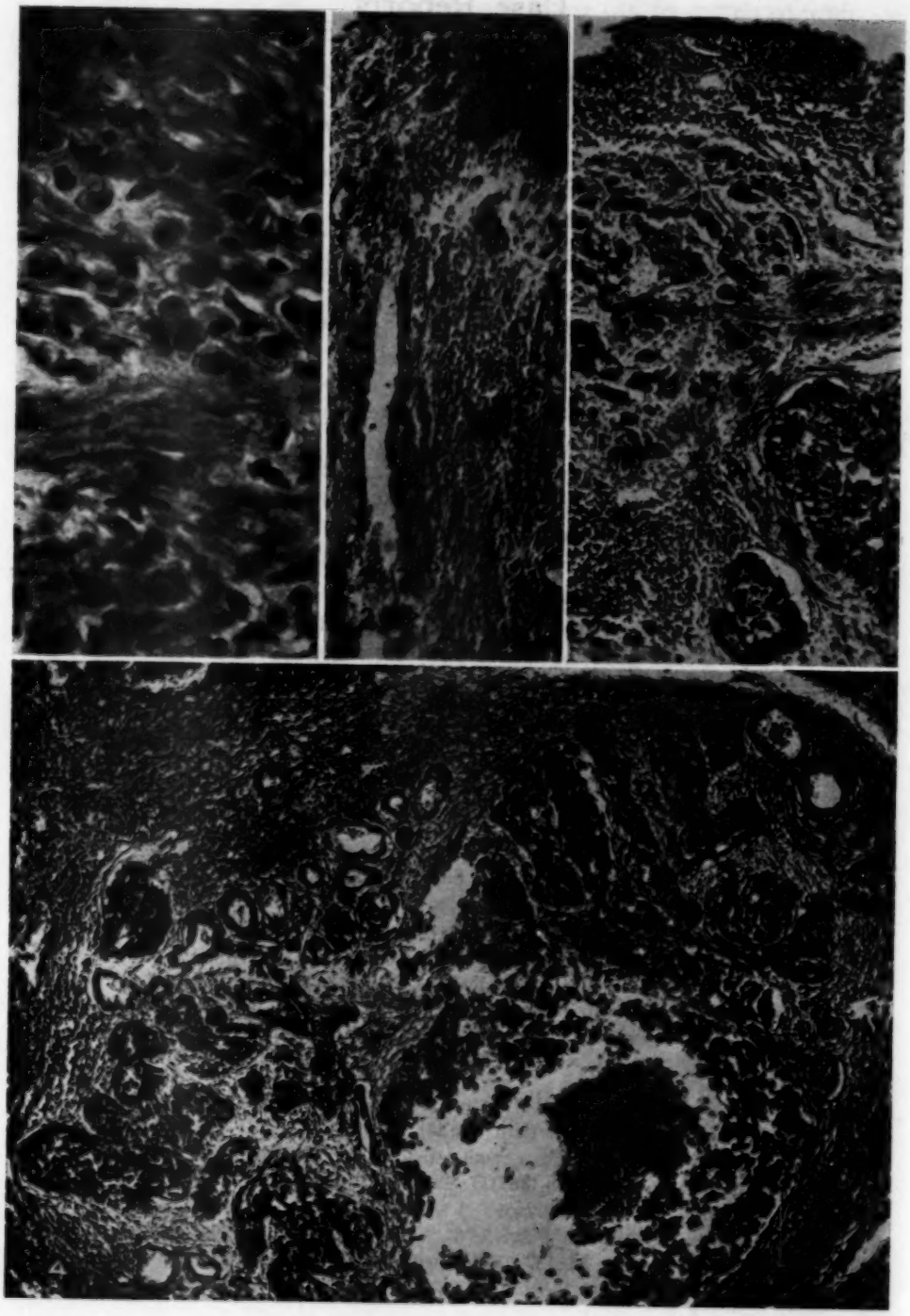
An 85 year old white man was admitted to the Metropolitan State Hospital, March 29, 1944, with a diagnosis of senile psychosis (paranoid type). In the last few months he had become restless, confused and assaultive, with persecutory delusions. His mental status did not change during his stay in the hospital. The medical history was not significant except for an operation on the prostate ten years before. On admission he was poorly nourished and had slight hypertension and general arteriosclerosis.

The hemoglobin content was 94 per cent (Sahli); the red blood cell count was 4,830,000 and the white cell count 6,600; the Hinton test was negative. In December 1944, during an infection of the respiratory tract, the hemoglobin content was 70 per cent (Sahli) and the red blood cell count 3,800,000. Soon afterward he refused to take food, although he expressed no specific complaints. He started to lose weight and died from a terminal infection of the respiratory tract, Feb. 18, 1945.

Autopsy (nineteen hours after death).—The cause of death was confluent bronchopneumonia of both lungs with fibrinous pleuritis. Cerebral and general arteriosclerosis, nephrosclerosis, and hemorrhagic cystitis were also present. Unusual and unexpected were the findings in the stomach. This organ was

From the Laboratory of the Metropolitan State Hospital.

1. Pasternack, J.: Am. J. Path. **11**:541, 1935.
2. Herxheimer, G.: Beitr. z. path. Anat. u. z. allg. Path. **41**:348, 1907.
3. Wood, D.: Arch. Path. **36**:177, 1943.
4. Martin, J. F., and Pollosson: J. de méd. de Lyon **17**:553, 1936.
5. Scheffler, M. M., and Falk, A. B.: Am. J. Cancer **38**:359, 1940.
6. Takagi, C.: Gann **31**:137, 1937; cited by Wood.[3]



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empty. The wall was thickened. From the mucosal surface numerous small grayish nodules protruded into the lumen. The thickening of the wall and the nodules extended from the pyloric region through the fundus and both curvatures to the cardia. The thickening of the wall was irregular. Between the cardia and the pylorus there were several areas in which the wall was normal in thickness and appearance. On the cut surface the walls showed two different layers, well demarcated from each other. The inner layer was thicker. It was white and firm and consisted of hypertrophic mucosa and submucosa. The outer layer was grayish red and consisted of the muscular tunics and the serosa. In most places the entire wall measured from 6 to 8 mm. while the inner layer was from 3 to 5 mm. thick. The wall in the prepyloric region was the thickest. Here it measured 11 mm., while the inner layer was 7 mm. thick. A few enlarged, firm, grayish periportal lymph nodes and two small nodules in the liver proved the neoplastic nature of the process. The cardia, the lower part of the esophagus and the small and the large intestine were grossly normal. There were no metastases elsewhere.

Microscopic Examination.—Sections from different thickened areas of the gastric wall gave essentially the same picture. Normal mucous glands were occasionally seen, but in most of the areas the mucosa was replaced either by accumulations of round cells or by long columns of pale polymorphous large cells with eccentric nuclei. The size and the shape of these pale cells varied to a considerable degree. The mucosa was separated from the submucosa by muscularis mucosae. The submucosa consisted of a thick layer of connective tissue fibers with many blood vessels and scattered accumulations of round and plasma cells. The lymphatic spaces were infiltrated by columns of the same large polymorphous pale cells with eccentric nuclei, which showed a glandular and alveolar arrangement in some places. In other areas, such cells had an anaplastic appearance. Similar cells infiltrated the spaces between the muscle bundles and the perineural spaces. Both muscle layers were hypertrophic and contained an increased amount of connective tissue. The tumor cells showed mitotic figures in different areas (fig. 1). The area of the greater curvature 3 cm. distant from the cardia was the only one where a quite different picture of the tumor was observed. Grossly, the thickened area here looked similar to other thickened areas. Microscopically, however, there was a sudden transition at the cardiac edge of the tumor from normal glandular mucosa to a mucosa formed by solid layers of large hyperchromatic polygonal cells with large dark nuclei. Some of the cells were flat, others were elongated with oblong nuclei, and others were almost round with round nuclei. The cells and their nuclei

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EXPLANATION OF PLATE.

Fig. 1.—Infiltration of the submucosa by polymorphous tumor cells with eccentric nuclei, accompanied by round cells. Hematoxylin and eosin; × 391.

Fig. 2.—Mucosa covered by squamous epithelial cells. Fibroplastic proliferation is seen in the submucosa. Hematoxylin and eosin; × 187.

Fig. 3.—Section from the same area as figure 2. Squamous cell carcinomatous infiltration of the submucosa. Some of the tumor cells have a basal cell character. Hematoxylin and eosin; × 188.

Fig. 4.—Same area of the submucosa as that in figures 2 and 3. Displaced mucous glands with adenocarcinoma may be seen. Hematoxylin and eosin; × 188.

varied in size and shape. Some showed a whorl-like arrangement but no glandular structures. Cell masses invaded the submucosa and the submucosal vessels. Their appearance in some places was that of basal cell carcinoma, although most of the cells presented the picture of squamous cell carcinoma with cornified epithelial pearls and keratinizations (fig. 3). Accumulations of round cells replaced parts of the mucosa and extended between the tumor masses into the hypertrophic submucosa, which consisted of a thick layer of connective tissue fibers. A small area of the mucosa was covered by normal squamous epithelial cells, which in one region had changed their character. The cells there became more irregular and hyperchromatic and invaded the submucosa in solid masses (figs. 2 and 3). In the submucosa of this area displaced mucous glands were lined by cuboidal epithelial cells, their lumens were distended and they were surrounded by clusters of round cells. Some of the glandular cells had proliferated and formed several layers of hyperchromatic cells with dark nuclei. The lumen and the glandular structure were still visible. However, the picture was definitely that of adenocarcinoma (fig. 4). In other glands, the lumen was obliterated, and the glandular structure had disappeared and was replaced by solid layers of hyperchromatic, polygonal cells with large nuclei. Cornified epithelial pearls were seen similar to those observed in the tumor of the mucosa of this region.

The cardia and the few areas of the gastric wall which grossly appeared normal showed microscopically normal glandular mucosa, submucosa and muscularis. There were some accumulations of round cells between the mucosal glands and in the submucosa.

Only a small amount of lymphoid tissue was visible at the edge of an involved periportal lymph node. Most of the node consisted of solid layers of large irregular hyperchromatic cells with large dark nuclei. These cells varied in size and shape; some were rounded, some were elongated and some were polygonal and irregularly shaped. The center of the node was largely necrotic. A number of mitotic figures and many cornified epithelial pearls were seen. The solid layers of tumor cells alternated with bundles of connective tissue fibers containing clusters of round cells.

The liver cells adjacent to a tumor node contained many fat droplets and brownish pigmented granules. The tumor nodules consisted of solid layers of polygonal hyperchromatic cells of varying size and shape with large dark nuclei. Mitotic figures and many cornified epithelial pearls were seen. Connective tissue fibers were observed between the layers of tumor cells; some of the tumor cells showed a whorl-like arrangement. The network of connective tissue contained accumulations of round cells and histiocytes with hemosiderin.

Nothing remarkable was observed in other organs except arteriosclerotic changes.

COMMENT

Several explanations have been given as to the cause of heterologous adenoacanthoma, or adenocancroid, of the stomach. According to Herxheimer,[2] the mixed glandular and squamous carcinomatous cells were derived from undifferentiated embryonic cell groups in the gastric mucosa rather than from displaced esophageal cells. Krompecher,[7] on the other hand, expressed the belief that nonembryonic undiffer-

7. Krompecher, E.: Beitr. z. path. Anat. u. z. allg. Path. **72**:163, 1924.

entiated basal cells, normally present in the gastric mucosa, developed into the cells of squamous and glandular carcinoma. Following this theory, Wood [8] expressed the opinion that the carcinogenic agent, whatever it was, stimulated these basal cells to proliferate along two different lines leading to squamous and glandular cells. The possibility of true metaplasia of normal mucosal cells under certain conditions preceding the neoplastic proliferation or occurring at the same time was emphasized by Lubarsch.[8] Oberling [9] and associates explained adenoacanthoma of the stomach as a metaplasia of adult cells in 1 instance and as congenital heterotopy in another, reported by Oberling later.

The attempts to produce squamous cell metaplasia of gastric mucosa in animals were only occasionally successful (Fuetterer [10]; Klein and Palmer [11]; Howes and Vivier [12]). Recently, however, Stewart and Lorenz [13] succeeded in producing adenocarcinoma and adenoacanthoma in the forestomach and in the pyloric stomach of mice with injections of methylcholanthrene. They concluded from their experiments that under carcinogenic influences a normal glandular mucosa could differentiate into glandular and squamous cell neoplastic elements.

The case of adenoacanthoma reported in this paper is different from those described in the literature.[14] The tumor had not been suspected during life, although at autopsy it was found that large areas of the gastric wall from the pylorus to the cardia were involved in a neoplastic process preceded or accompanied by chronic hypertrophic gastritis. The tumor was predominantly a more or less anaplastic type of adenocarcinoma, infiltrating all layers of the wall. The adenocarcinomatous cells were of an irregular pale polymorphous type with eccentric nuclei. The part of the tumor which consisted of both adenoacanthomatous and squamous cell elements was limited to a small circumscribed area of the greater curvature near the cardia, covered by squamous epithelium similar to that normally present in the esophagus. There was no continuation of the squamous epithelium from this region to the cardia. There are two possibilities to explain the presence of this squamous epithelium. It could represent congenital heterotopy, a displacement

8. Lubarsch, O.: Verhandl. d. deutsch. path. Gesellsch. (1906), 1907, p. 198.

9. Oberling, C., and Wolf, M.: Bull. Assoc. franç. p. l'etude du cancer **16**:68, 1927. de Martel, T.; Oberling, C., and Pernet, J.: ibid. **19**:470, 1930.

10. Fuetterer, G.: Ergebn. d. allg. Path. u. path. Anat. **9**:706, 1903.

11. Klein, A. J., and Palmer, W L: Arch. Path. **29**:814, 1940.

12. Howes, E. L., and Vivier, P. J.: Am. J. Path. **12**:689, 1936.

13. Stewart, M. L., and Lorenz, E. J.: J. Nat. Cancer Inst. **2**:193, 1941; **3**: 175, 1942.

14. Bödecker, F.: Ztschr. f. Krebsforsch. **24**:406, 1927. Pasternack[1] Herxheimer.[2] Wood.[3] Martin and Pollosson.[4] Scheffler and Falk.[5] Takagi.[6] Lubarsch.[8] Oberling and others.[9]

of esophageal epithelium. It seems more likely, however, that the squamous epithelium was derived from undifferentiated basal cells in the gastric mucosa which had undergone metaplastic change after a chronic inflammation of the stomach. The patient's age (86 years) and the presence of chronic hypertrophic gastritis make the latter conclusion more probable. Some of the squamous cells had become neoplastic and formed a squamous cell carcinoma with cornified epithelial pearls invading the submucosa. Some parts of the tumor in this region had the appearance of basal cell carcinoma and originated probably from undifferentiated basal cells. Epithelial cells of displaced mucous glands in the hypertrophic submucosal layer of the same region had proliferated into a mixed adenoacanthoma with glandular and squamous cell carcinomatous structures. Such displaced mucous glands associated with gastric carcinoma have been found not infrequently in the stomachs of elderly people (Lubarsch [8]). It is interesting to note that the metastases of the liver and the periportal lymph nodes showed pure squamous cell carcinoma.

It is difficult to decide in which area of the stomach the neoplastic process started. However, it can be stated that the carcinogenic agent, whatever it was, had stimulated epithelial cells of the gastric mucosa and of mucous glands to proliferate along three different lines. First, the glandular mucosa of large parts of the stomach from the pylorus to the cardia had developed into diffuse adenocarcinoma invading all layers of the stomach wall. Second, metaplastic or heterotopic squamous epithelium covering a small area of the stomach near the cardia had proliferated into squamous cell carcinoma. Because of the admixture of basal cell carcinomatous elements, undifferentiated basal cells probably participated in the formation of this part of the tumor. Third, epithelial cells lining displaced mucous glands in the submucosa of the same region had formed an adenoacanthoma with mixed glandular and squamous cell carcinomatous elements.

Heterotopic or metaplastic squamous epithelium covering the gastric mucosa in association with adenoacanthoma has not been reported in any other case of adenoacanthoma of the stomach; in all other cases noncancerous squamous cell metaplasia was consistently absent (Wood [3]).

SUMMARY

Adenoacanthoma of the stomach was observed associated with chronic hypertrophic gastritis at autopsy of a man 86 years old. It was predominantly more or less anaplastic and extended from the pyloric region to the cardia. The squamous cell carcinomatous and adenoacanthomatous element was limited to a small area of the greater curvature near the cardia covered by metaplastic or heterotopic squamous epithelium.

This epithelium had proliferated into squamous cell carcinoma with admixture of basal cell elements deriving from undifferentiated basal cells. A mixed type of adenoacanthoma had been formed by epithelial cells lining displaced mucous glands in the same region. Different epithelial elements, glandular mucosal cells, basal and squamous cells had been stimulated under a carcinogenic influence and had differentiated into mixed carcinomatous elements. The metastases in the liver and in regional lymph nodes showed pure squamous cell carcinoma with many cornified pearls.

Notes and News

Appointments.—George M. Hass, chief of pathology in the School of Aviation Medicine, Randolph Field, Texas, has been appointed professor of pathology in the Rush-Presbyterian Hospital Division of the University of Illinois College of Medicine.

D. A. Mactadyen, chief of the division of chemistry and physics at the Army Medical Center, Washington, D. C., has been appointed professor of biochemistry in the Rush-Presbyterian Hospital Division of the University of Illinois.

Robert E. Stowell, assistant professor of pathology in the Washington University School of Medicine, St. Louis, has been awarded a fellowship by the Commonwealth Fund of New York to enable him to study in Sweden under Dr. Torbjorn Caspersson, chief of the Swedish Institute for Cytologic Research.

Lieutenant Colonel Elson B. Helwig, who has been in the Army of the United States for the past three years, has returned to the department of pathology of Washington University School of Medicine.

Deaths.—Mazyck Porcher Ravenel, first bacteriologist of the Pennsylvania State Livestock Board, died January 14, aged 84 years.

Awards.—Brigadier General Stanhope Bayne-Jones, deputy chief of the Preventive Medicine Service, Office of the Surgeon General, was recently awarded the Distinguished Service Medal for his outstanding "contribution to the maintenance of health within the Army. As administrator of the Epidemiological Board, he directed the extension, administration and military application of the worldwide research and control program conducted by this board and its ten commissions." Before taking up his military duties in 1942 General Bayne-Jones was professor of bacteriology at Yale University.

The John Scott Award of the Philadelphia Board of City Trusts has been given to Ernest W. Goodpasture, professor of pathology and dean of the Vanderbilt University School of Medicine, Nashville, Tenn., for his "development of a method for the cultivation of viruses that have made hitherto unknown vaccines possible." Edwin J. Cohn, professor of biochemistry, Harvard Medical School, Boston, received a similar award for developing "a substitute for dried blood plasma used in the treatment of shock in the war."

Society News.—The Society of American Bacteriologists announces the election of the following officers to serve in 1946: James Craigie, Toronto, Canada, president; Thomas Francis Jr., Ann Arbor, Mich., vice president; Leland W. Parr, Washington, D. C., secretary-treasurer; M. J. Rosenau, Chapel Hill, N. C., and Frederick Smith, Montreal, Canada, councilors.

Friends of Medical Research.—This group has been organized under the auspices of the New York Academy of Medicine to inform the public about animal experimentation and what such experimentation has contributed to science and health. Simon Flexner has been named honorary president. The first efforts are directed toward the defeat of the DiCostanzo-Davidson bill in the legislature of New York, which proposes to make research work with dogs unlawful.

Book Reviews

Pulmonary Tuberculosis in the Adult: Its Fundamental Aspects. By Max Pinner, M.D., chief of the division of pulmonary diseases, Montefiore Hospital for Chronic Diseases, New York; clinical professor of medicine, College of Physicians and Surgeons, Columbia University, New York. Price, $7.50. Pp. 579, with 59 illustrations. Springfield, Ill.: Charles C Thomas, Publisher, 1945.

In this extremely well written and thoughtful book Dr. Pinner presents the rich fruits of his long experience with tuberculosis as a clinician and as a devoted and constantly inquiring student of the disease. It is not his purpose in this book to give directions for diagnosis and treatment but rather to present a synthesis of the multiple factors that bear on phthisiogenesis, in order to provide a basis for the understanding of the disease and a foundation for practical work. The result is a treatise that cannot fail to be of the greatest interest and importance to all who are concerned with the problem of pulmonary tuberculosis in the adult. The clinical aspects are closely and admirably correlated with the underlying pathologic alterations. Few clinical students of tuberculosis have as sound and as clear an understanding of the pathology of the disease as has Dr. Pinner, and his clinico-pathologic correlations are masterful. No less masterful are the chapters that outline the principles bearing on incipient tuberculosis and on collapse therapy. While it is not to be expected that all of any person's views on controversial matters pertaining to therapy will be shared by every one who deals with tuberculosis, still there speaks here a deeply thoughtful and keenly observant clinical student of the disease, from the vantage point of an abundant and well used experience.

While the portions of the book dealing with the clinical and the morphologic aspects of tuberculosis, with which Dr. Pinner is thoroughly experienced at first hand, are superb, in the portions devoted to fundamental immunologic principles and to the interpretation of experimental studies the author treads less familiar ground, and the treatment is correspondingly less ably critical, less logical and too often colored by bias and by unwarranted dogmatism. In his preface he states that his guiding principle in the writing of this book was to achieve consistency in all of its aspects, in order to present a plausible picture, rather than to present the available evidence impartially, that he therefore expects the criticism of personal bias and that he regards that as a small price to pay for coherence and clarity. The wisdom of that procrustean attitude in dealing with matters of science must be questioned. At all events, in view of it and of the fact that statements supporting his own views are often presented with a dogmatic finality that is contrary to the existing evidence, it will be well to bear his warning preface constantly in mind. In his discussion of basic immunologic principles, the presentation of pertinent evidence, which often exists even in the papers quoted, appears to be sacrificed in the interests of maintaining the consistency of his own views. Since it would be altogether unfair for the reviewer to make these statements without documenting them, the following examples have been selected:

On page 111, in support of the view that resistance is determined mainly by the degree of local inflammatory reaction, Dr. Pinner quotes a study on animals with different degrees of native resistance as demonstrating that "invariably, the resistant animals showed the quickest response" to a local, intracutaneous injection of killed tubercle bacilli. Column I of table 4 in the article referred to shows that this was not the case. There was no difference between the reactions of normal animals of the most resistant and of the most susceptible groups, nor is it claimed in the paper that there was.

On page 104 he cites the work of one investigator as evidence that in experimental reinfection the tubercle bacilli are destroyed without any preliminary multiplication. He fails to mention that this was not confirmed by subsequent equally competent investigators.

On page 107 he states that it has been "unequivocally shown" that the phagocytosis of tubercle bacilli by macrophages is not enhanced by immune serum. The

reverse, however, has long been well established, and the reviewer knows of no documented studies to the contrary.

In the same discussion it is stated as an "incontestable fact" that passive transfer of acquired resistance in tuberculosis "is impossible." It is not pointed out that no adequately controlled study of the matter has yet been carried out, nor that in the better controlled of the existing studies evidence of passive transfer of resistance was obtained.

On page 94 it is stated that when a massive dose of nonvirulent bacilli is injected subcutaneously an abscess develops, there is limitation of spread of the bacilli and healing occurs. The limitation of spread and the suppression of the bacilli are attributed to the acute inflammation. The post hoc logical fallacy is obvious; and the author does not mention the fact that a similar injection of virulent bacilli will likewise cause an abscess but that in this case widespread devastating disease results. The outcome in the case of the nonvirulent bacilli is clearly a result of the nature of the bacillus and not of the inflammatory reaction.

On page 98 it is stated as an "incontrovertible fact" that tuberculous tissue is required for the acquisition of resistance in tuberculosis. It is true that up to the present resistance has been acquired only on the introduction of entire bacilli into the body and that the complex bacillus causes the development of tuberculous tissue. Here again, however, the author falls into a post hoc logical fallacy by concluding that the tuberculous tissue itself is necessary for the acquisition of resistance. In the present state of information it would certainly be a great mistake to regard that as an "incontrovertible fact." Highly potent antigens that immunize in the absence of inflammatory tissue reactions have been isolated from the pneumococcus and from other bacteria, and there is good reason to believe that the same end may be accomplished in the case of the tubercle bacillus.

On page 118 the author states dogmatically that it has never been demonstrated that anything more than cutaneous reactivity can be abolished by desensitization. He does not mention the thoroughly controlled studies of the numerous investigators who have demonstrated clearly that systemic reactivity is also abolished by desensitization.

Indeed, throughout the discussion of hypersensitivity and immunity the author's bias leads him into unfortunate inconsistencies, inaccuracies and logical faults. Thus he flatly rejects the work of the many investigators who have shown that tuberculous animals can be desensitized without loss of their immunity, insisting dogmatically that "no tuberculous animal has, as yet, been 'desensitized' or deprived of its allergy in any valid sense of the term" (page 118). When, however, he comes later to discuss one paper in which, in conformity with his own view of the significance of allergy, the experimental abolition of hypersensitivity was regarded as deleterious (page 146), he readily accepts those animals as having been desensitized and the work as being "fundamentally important," though the method, the criteria and the degree of desensitization in that experiment were precisely those used by the many other equally competent investigators whose opposite results he rejects. Furthermore, he does not mention that this same author, whose conclusions regarding a deleterious effect of desensitization in experimental animals he accepts, subsequently reported studies from which it was concluded that the desensitization of tuberculous patients was not deleterious but beneficial.

It should be clearly understood, however, that the aforementioned inadequacies in the treatment of immunologic principles and experimental data do not apply to the book as a whole. The more that Dr. Pinner knows by first hand experience of any aspect of tuberculosis about which he writes, the less dogmatic he becomes, and the more sound and balanced is his treatment of the subject; by far the greater part of the book falls within his own province of experience. It is only to be regretted that a book which in its clinical and pathologic portions is without doubt one of the finest and most valuable ever written on pulmonary tuberculosis should have been marred by capricious treatment of any of its fundamental sections.

The book is admirably illustrated and beautifully published.